ABSTRACT BOOK

INDEX

SYMPOSIUM LECTURES abstracts p. 2
KEYNOTE LECTURES abstracts p. 22
ORAL PRESENTATIONS abstracts p. 25
POSTER PRESENTATIONS abstracts p. 180

Please use the SEARCH function to search for final ID number, author(s), and keyword(s)
SYMPOSIUM LECTURES abstracts

Q005
Periodontal Infectogenomics: Does the Host Genome Affect the Subgingival Microbiota?
Luigi Nibali
King’s College, London, United Kingdom
We now understand that probably around a third of the variance of periodontitis in the population is attributable to heritable factors. However, after more than 20 years and several small-scale and large-scale studies, the search for the genetic factors predisposing to periodontitis continues. What has clearly emerged is that this predisposition is, at least partially, mediated by the subgingival microbiota. In this context infectogenomics defines the effect of host genetic variants on influencing the growth of specific microbes and hence the composition of microbial biofilms. This talk will shed light into this topic and will describe the evidence gathered so far, with an eye to possible future applications.

Q006
Application of Artificial Intelligence to Head and Neck Pathology.
Ali Khurram
University of Sheffield, Sheffield, United Kingdom
Artificial Intelligence (AI) has become an integral part of our daily lives and its use is becoming very popular in healthcare settings due to developments in computational technology and methods. AI can aid diagnostics by removing subjectivity, using automation and quantification to provide more objective and quantifiable information which can guide patient diagnosis and treatment. However, it’s use in head and neck oral pathology remains limited. This talk will introduce AI and relevant methods and terminologies to the audience and provide an overview on its reported use in head and neck pathology in the literature to date. Furthermore, it will share recent ground-breaking work and findings from Dr Khurram’s research group (NEOPATH-neopath.org.uk). The potential for AI to be used as diagnostic and prognostic aid in cancer and pre-cancer and how it can transform patient care will also be discussed as well as future directions.

Q007
Physical Properties and Characterization.
Amre Atmeh
MBRU, Dubai, United Arab Emirates
With the growing popularity of hydraulic calcium silicate cements and the expanding scope of their clinical applications in dentistry, it has become essential to understand their properties and characteristics. Such understanding can bridge the gap between the anticipated potentials of these materials and their actual performance in-situ. This lecture aims to provide a glance about the available scientific evidence related to the physical properties of hydraulic calcium silicate dental cements, relating these properties to the clinical performance. Hydraulic calcium silicate cements represent a family of materials that sets in the presence of water. Their setting reaction is basically a hydration reaction that produces a matrix of calcium silicate hydrate and ion-rich solution principally composed of calcium hydroxide. Despite the simple description, this process is much complex that involves more interfering factors, which may affect the behaviour of the hydrating cement. This grants HCSCs an interactive nature allowing them to exchange ions and fluids with the surrounding environment, and adjacent substrates such as dentine. Hence, the cements’ properties are dictated by the environment that is related to the clinical conditions under which we apply these cements. The evidence discussed in this lecture is based on a comprehensive review of the scientific literature related to the physico-chemical properties of these cements. This will be discussed along with the interfacial properties of HCSCs with dentine paving the way for further discussion about the biological and clinical evaluation of these cements in the following lectures of this symposium.
Biological Evaluations.
Josette Camilleri

University of Birmingham, Birmingham, United Kingdom

Hydraulic cements are a group of materials which hydrate in contact with water and also interact with environmental fluids. They are used almost exclusively for endodontic procedures with the same materials being used for a range of procedures. The main features of hydraulic cements with tricalcium silicate chemistry are the formation of calcium hydroxide on setting and the interaction of these materials with the clinical environment. This interaction is dependent on the location that the material is placed. There are a number of materials currently available for clinical use with variable evidence of their efficacy and mode of action. Regardless of the specific material properties and interactions, clinicians use hydraulic cements with the same clinical protocol as that used for materials with different chemistries. For most clinical procedures, the scientific evidence for the clinical protocol adopted is not always present since most clinical techniques are adopted and descended in different generations without any research undertaken to validate their effectiveness.

Biological studies are important to test the interaction of a material with the host tissues. Biological studies are conducted at the cellular and tissue level and also the effect of the specific regime used on the elimination of bacteria is assessed.

The aim of this symposium is to evaluate the scientific evidence for the biological studies undertaken for hydraulic calcium silicate cements used for different procedures in Endodontology and whether this influences the clinical use of these materials.

Clinical Evaluation.
Nastaran Meschi

KU Leuven, Leuven, Belgium

Objectives:
To assess the current clinical endodontic status of hydraulic calcium silicate cements and to identify future perspectives.

Methods:
The literature was reviewed using PUBMED. For each clinical endodontic use of hydraulic calcium silicate cements, the clinical data were extracted and evaluated, based on the classification by Camilleri: intra-coronal, intra-radicular and extra-radicular.

Results:
From the 151 records identified, 51 met the eligibility criteria. Hydraulic calcium silicate cements have been mostly applied in vital pulp therapy (37 publications in the intra-coronal section), followed by root-end surgery (8 publications in the extra-radicular section) and subsequently intra-radicular in immature permanent teeth (6 publications).

Hydraulic calcium silicate cements:
do perform well for all endodontic treatment modalities and are comparable or superior to previous materials;
per endodontic treatment modality, the different hydraulic silicate cements applied, result in comparable (favourable) outcomes;
the most prominent adverse event is discolouration due to bismuth oxide in MTA;
there is lack of consensus regarding restoration (coronal/intracanalicular);
the limitations in clinical trials are mainly investigator and/or operator related.

Conclusions:
Despite limitations, the hydraulic cements have made a difference in clinical outcomes in endodontics. Nevertheless, the clinical protocols need to be updated to enable the materials to be employed effectively.
Cross-Linked Hyaluronic Acid: Biologic Background and Clinical Outlook in Periodontal Therapy.

Anton Sculean

University of Bern, Bern, Switzerland

Hyaluronic acid (HyA), an anionic, non-sulfated glycosaminoglycan structured biomolecule, is a major natural component of the extracellular matrix in many tissues including the skin, joints, eyes, and periodontium. Physiochemically and biologically, HyA is hygroscopic, viscoelastic, bacteriostatic, antibacterial, anti-inflammatory, and anti-edematous. It significantly stimulates clot formation, induces angiogenesis, and increases osteogenesis.

A novel high molecular, cross-linked formulation (xHyA) has recently been shown to enhance the proliferative, migratory and wound healing properties of cell types involved in soft tissue wound healing following regenerative periodontal surgery, induces the growth of osteoprogenitors and maintains their stemness, thus potentially regulating the balance between self-renewal and differentiation during bone regeneration following reconstructive oral surgeries.

The aim of the present symposium is to summarize, in three consecutive lectures, the current biologic and clinical evidence on the use of xHyA in various oral reconstructive surgical procedures.

The available evidence from preclinical and clinical studies indicates that:

xHyA promotes periodontal wound healing/regeneration in intrabony-, recession and furcation defects and improves the outcomes of regenerative surgery in intrabony defects and recession coverage procedures.

The combination of xHyA with various types of bone grafting materials may additionally improve the outcomes of regenerative periodontal surgery in complex clinical scenarios.

Very recent clinical observations have also pointed to the potential clinical benefit of using xHyA in Guided Bone Regeneration (GBR).

Periodontal Regeneration in Intrabony Defects With Combined Approach of Cross-Linked Hyaluronic Acid and Xenograft.

Darko Bozic

University of Zagreb, Zagreb, Croatia

Hyaluronic acid (HyA), an anionic, non-sulfated glycosaminoglycan structured biomolecule, is a major natural component of the extracellular matrix in many tissues including the skin, joints, eyes, and periodontium. Physiochemically and biologically, HyA is hygroscopic, viscoelastic, bacteriostatic, antibacterial, anti-inflammatory, and anti-edematous. It significantly stimulates clot formation, induces angiogenesis, and increases osteogenesis.

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Is Cross-Linked Hyaluronic Acid a Successful Molecule for a Successful GBR?
Georges Khoury
Hôpital Rotshild, Paris, France

Hyaluronic acid (HyA), an anionic, non-sulfated glycosaminoglycan structured biomolecule, is a major natural component of the extracellular matrix in many tissues including the skin, joints, eyes, and periodontium. Physiochemically and biologically, HyA is hygroscopic, viscoelastic, bacteriostatic, antibacterial, anti-inflammatory, and anti-edematous. It significantly stimulates clot formation, induces angiogenesis, and increases osteogenesis.

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Collagen Sets the Foundation for Bone Vascularization and Regeneration.
Imad About
Aix-Marseille University, Marseille, France

In critical size defects, bone regeneration is facing the real challenge of inducing rapid vascular ingrowth and bone marrow stem cell recruitment.

Dual-phase bone substitutes are frequently used as therapeutic scaffolds for post-extraction ridge preservation and for bone regeneration in critical-size bone defects.

In order to understand how this type of materials is involved in bone regeneration, we investigated the angiogenic potential of xenogenic dual-phase bone substitutes containing native collagen of porcine and equine origins and their effects on bone marrow mesenchymal stem cell recruitment.

Our data showed that the application of these xenogenic substitutes significantly enhanced the release of Complement Bioactive C5a molecule from injured periodontal ligament cells (PDL) which induced bone stem cell proliferation and recruitment. These xenogenic materials also significantly induced angiogenic growth factors release from PDL cells, enhanced endothelial cell proliferation as well as angiogenesis as demonstrated by an increased formation of capillary-like structures. Our study also demonstrated that stem cell proliferation and recruitment was significantly higher with collagen-containing materials than without collagen.

Taken together, xenogenic bone substitutes which contain native collagen, provide structural and biological support for vascularization and bone regeneration by enhancing neo-angiogenesis and mesenchymal stem cell recruitment. These are major pre-requisites for bone regeneration in critical size defects.
Q014
Ridge Preservation With Collagenated Biomaterials.
Antonio Barone
School of Dental Medicine, Department of Surgical, Medical, Molecular Pathologies and of Critical Needs, University of Pisa, Pisa, Italy
Alveolar bone crest undergoes dimensional ridge changes after tooth extraction, these changes cannot be completely counteracted, even though a proper management could allow the achievement of the best possible outcome for the patient. The ridge preservation technique is known to be effective on the reduction of alveolar bone resorption after a tooth extraction. The objective of this lecture is to illustrate the surgical techniques and the biological principles that should be followed to obtain the better clinical outcomes. The fresh extraction socket classification will be discussed. The advantage and downsides with immediate implants and ridge preservation procedures will be discussed and analysed. The flapless and flapped techniques to perform socket preservation is still considered a matter of discussion in the scientific community. The flapless ridge preservation technique showed to be as effective as the flapped technique, since the bone healing was similar within the two groups; on the other hand, the flapless procedure seems to offer a less postoperative discomfort, better preservation of keratinized gingiva and stability of muco-gingival junction. The immediate implants showed several advantages, even though in the esthetic area the use of a connective tissue graft seems to offer a good solution in term of mid-facial mucosa recession.

Q015
Immediate Implant Restorations With the Multi-Layer-Technique - 5 y Results.
Hannes Wachtel
Implaneo Dental Clinic, München, Germany
The esthetic immediate implant placement and restoration in compromised post extraction sites requires the combination of established surgical techniques: atraumatic tooth extraction with maximum preservation of the surrounding tissues followed by soft tissue augmentation with a sub-epithelial connective graft and hard tissue augmentation using the bone lamina technique and cortico-cancellous bone substitutes. Implant site development is the key factor when it comes to predictable results in the esthetic zone. The Multi-Layer Technique enables in only one surgical intervention a minimally invasive approach to immediate implant restorations. A 5 year follow up of consecutively treated cases demonstrates stable results with excellent tissue architecture.

Q016
Oral and Systemic Health - is There a “new” Link With COVID-19?
David Herrera
Complutense University of Madrid, Madrid, Spain
During the last decades, the association between periodontitis and different systemic conditions has been established, based on epidemiological studies, preclinical studies to assess biological plausibility and interventional studies. Today, the associations have been clearly defined with a variety of conditions, highlighting endocrine conditions (including diabetes mellitus), cardiovascular diseases, adverse pregnancy outcomes, respiratory infections, rheumatoid arthritis, Alzheimer’s disease, among others. The bases for the association include the frequent bacteremia observed in periodontitis subjects, together with higher levels of systemic inflammation. The relevant role of the mouth in the transmission of SARS-CoV-2 and the severity of COVID-19 has recently raised the hypothesis of a possible association between periodontitis and the severity of COVID-19. Different studies have already explored the association of periodontitis and oral hygiene and different respiratory diseases (including chronic obstructive pulmonary disease, asthma, community-acquired pneumonia, obstructive apnea), suggesting a rationale to also explore the association between periodontitis and COVID-19. Although the number of published studies is still limited, and the quality of the evidence is also improvable, the initial evidence is suggesting that periodontitis may be associated with a more severe COVID-19 and even with higher risk of death due to COVID-19. The implications of these findings need to be cautiously interpreted, since they may be valid not just for SARS-CoV-2 and COVID-19, but also for other viral and bacterial infections in the upper and lower respiratory tract, and may further emphasize the importance of oral hygiene and periodontal health in systemic health.
Q017
Aerosols in the Dental Setting: a Misty Topic.
Fridus van der Weijden
ACTA, Amsterdam, Netherlands
Since the start of the COVID-19 pandemic an innumerable amount of (mis)information and advice on how to stay safe and prevent the spread of coronavirus has been published. Airborne transmission of various pathogens has been identified in community and healthcare settings. Also viral transmission of airborne droplets/particles that have settled on surfaces has also been demonstrated. Especially in dentistry, due to aerosol-generating procedures there are potentially various infection risks for patients and those working in this field. Studies have shown that microorganisms in the mouth and respiratory tract can be transported in these aerosols, splash, and spatter. These can contaminate the mucous membranes of the mouth, respiratory passages, and eyes of the dental care professional as well surfaces and materials exposed in the environment. As such, it is important to prevent disease transmission within the dental practice. What preventive measures can and have been taken to counteract this and what have we learned during the pandemic?

Q018
The Role of the Oral Cavity in SARS-CoV-2- and Other Viral Infections.
Dieter Hoffmann
Institute for Virology, Munich, Germany
SARS-CoV-2 enters the human body via the respiratory tract and the oral cavity. As other respiratory viruses SARS-CoV-2 is transmitted by droplets and smaller aerosols. When they are inhaled SARS-CoV-2 infects epithelial cells of the upper airways. A second port of entry has been confirmed in several studies: SARS-CoV-2 also replicates in oral mucosa-and salivary gland cells. These sites are also virus reservoirs that can infect other organs, e.g. lungs and gastrointestinal tract as well as other individuals. Viral loads in saliva can surpass $10^{10}$ Geq/ml and infectious viral particles have been detected.
Droplets quickly sink to surfaces and thus only contaminate air and surfaces within 1.5 to 2 m. Aerosols including infectious SARS-CoV-2 can stay in the air for several hours possibly contaminating the entire indoor room.
In acute infection SARS-CoV-2 can be detected in other organs than the respiratory tract, i.e. GI- and urinary tract and immune tissues. Even after mild COVID-19 symptoms sequelae can be found in heart, lungs kidneys and blood vessels. Usually they do not correlate to post-COVID symptoms. However there can be generalized complications as “multisystem inflammatory syndrome” or “long COVID”.
In the oral cavity SARS-CoV-2 causes taste loss and mucosal lesions. Various cell types can be infected in cell culture models.
As SARS-CoV-2 influenza viruses are readily detected in saliva and nasopharyngeal swabs during acute infections. However, influenza viruses are usually excreted around one week, whereas SARS-CoV-2 RNA can be detected in saliva and swabs longer.
Compared to respiratory viruses human papilloma viruses can infect mucosa epithelial cells chronically. A possible consequence are cancers of the oral cavity; they are detected with increased frequency in the last years. Recently HPV DNA in saliva has been described as a tumor marker.
Preprocedural Mouthwashes for Infection Control – an Update.

Fabian Cieplik
University Hospital Regensburg, Regensburg, Germany

Since the spread of SARS-CoV-2 and the associated COVID-19 pandemic, dental office personnel have been considered a particularly vulnerable professional group due to their proximity to patients. In addition to various other additional infection control measures (e.g., use of personal protective equipment) that have been rapidly implemented in dental offices, preprocedural mouthwashes have also been recommended by various professional associations and dental organizations. While their effect toward reducing numbers of bacteria and various other viruses in dental aerosols and saliva has been known for many years, there is still considerable debate as to whether preprocedural mouthwashes could reduce viral load and viral infectivity in the oral cavity of SARS-CoV-2-positive patients. In this regard, a variety of antiseptic compounds have been proposed, and numerous in vitro studies and clinical trials have been initiated to evaluate the efficacy of known oral antiseptics against SARS-CoV-2.

In addition, the number of mouthwashes being offered continues to increase, and some manufacturers are vehemently promoting their products for “corona prophylaxis”. The question of potential benefits of such preprocedural mouthwashes remains relevant, not only for the prevention of COVID-19, but also for other infectious diseases. This presentation will review the efficacy of various antiseptics, such as chlorhexidine digluconate or cetylpyridinium chloride, against SARS-CoV-2 in vitro, and summarize and evaluate the latest findings from clinical trials evaluating mouthwashes to reduce viral load and infectivity in SARS-CoV-2-positive individuals.

Commercial and Experimental Self-Adhesive Composites: Where are we at Today?

António H. Delgado,1,2
Centro de Investigação Interdisciplinar Egas Moniz (CiiEM), Monte de Caparica, Almada, Portugal, 2Division of Biomaterials and Tissue Engineering, UCL Eastman Dental Institute, London, United Kingdom

The search for simplicity has always accompanied the history and evolution of modern adhesive dentistry. This led to the creation of simpler, user-friendly systems over the years, that make the procedures easier for the clinician to undertake and the patient to endure. Self-adhesive composites (SACs) were developed and introduced in restorative dentistry to surpass current multi-step adhesive systems, which are not ideal in terms of technique sensitivity, chair time required and expenditure. The self-adhesiveness of SACs is mainly linked to them having functional monomers in their chemical composition, such as 10-MDP, 4-META or GPDM. This equips them with chemical adhesion potential, making them theoretically able to ionically bond to hydroxyapatite-rich dental substrates. However, simplicity pays its price. These materials show consistently poor performance in in vitro and in vivo research, when compared to the conventional approach. This significantly hampers their clinical breakthrough, which is yet to take place. On that account, optimization of these materials is urgently needed, and research is being devoted to improving current self-adhesive options. This lecture covers the properties and flaws of current commercial SACs, which provides a standpoint for the development of a new generation of SACs, able to meet clinical expectations and future demands. Furthermore, latest results and findings of experimental SACs that are under development, will be showcased in this lecture. Such research is fundamental for SACs to thrive, as these materials are desirable to all.

Single Versus Multi-Bottle Dental Adhesives: How Big Is the in-Between Gap?

Mohammed H. Ahmed
Department of Dental Biomaterials, Tanta University, Tanta, Egypt

While single-bottle dental adhesives are gaining more market-popularity, a counteracting increasing awareness over their decreased durability is also ongoing. However, the in-between gap is getting broader due to many factors other than number of application steps. In this presentation, we will discuss the additional technical, compositional, and clinical aspects that could affect the adhesive-dentin interfacial durability and its response to factors other than the number of the application steps, with a brief discussion over the latest research endeavors for resolving this gap.
Q022
The Future of the ART Technique in Light of Novel Remineralising Dental Restorative Materials.
Melissa Tiskaya, Saroash Shahid & Robert Hill
Queen Mary University of London, Barts & The London School of Medicine and Dentistry, Institute of Dentistry, Centre for Oral Bioengineering, Mile End Road, London E1 4NS, UK
Atraumatic restorative treatment (ART) is a minimally invasive technique that was introduced in the 1980s for underdeveloped countries and children. More recently, since the COVID-19 pandemic due to the lack of aerosol generation, this technique is now more widely used around the world in both children and adults to overcome patients’ fear of dental treatment and minimise the progression of dental caries. This technique involves removing soft carious lesions via hand instruments, whilst leaving behind the hard carious lesions and placing a restorative material to remineralise the caries and restore the tooth. Glass ionomer cements (GICs) is the most commonly used restorative material in ART due to their fluoride releasing capability, however they only degrade under acidic conditions and do not release significant calcium and phosphate ions. Other materials such as resin based composites, resin modified GICs and Giomers have also been used for this technique. More recently, bioactive glasses (BAGs) have been developed, which release beneficial ions such as calcium, phosphate and fluoride ions, neutralise the local pH and facilitate the formation of a fluorapatite layer, which is more acid resistant than hydroxyapatite. This makes BAGs a good candidate to be used as fillers in dental composites. This lecture will explore novel remineralising dental restorative materials (i.e: BAG containing composites) that have the potential to be used in ART

Q023
Tissue Thickening With an Acellular, Porcine Dermal Matrix Over Dental Implants and Around Teeth – Clinical and Immunohistological Results.
Tobias Fretwurst
Regenerative Oral Medicine Section, Dept. of Dentistry, Oral and Maxillofacial Medicine,. University of Freiburg, Freiburg, Germany
For soft tissue grafting a wide range of techniques and materials are available. The autologous connective tissue graft represents currently the gold standard for tissue thickening. However, recently an acellular, porcine dermal matrix became available for soft tissue augmentation in periodontal and implant surgery. In this talk, principles of soft tissue grafting will be introduced and the latest data on soft tissue grafting with an acellular, porcine dermal matrix over dental implants and for recession coverage presented. What do we know about the immunological incooperation capacity of the matrix? What clinical results can be expected from recession coverage with the matrix in combination with a coronally tunnel technique? Where are the borders and limitations? This lecture is intended to provide a glimpse on the first available clinical scientific data.
Q024

Effects of Three-Dimensional Collagen-Based Matrices on the Regenerative Potential of Primary Human Oral Fibroblasts, Primary Human Periodontal Ligament Cells, and Pre-Osteoblasts.

Maria Asparuhova1, Anton Sculean2

1Laboratory Cell Biology, School of Dental Medicine, University of Bern, Bern, Switzerland, 2University of Bern, Bern, Switzerland

Xenogeneic three-dimensional (3D) collagen matrices may represent an alternative to autologous soft tissue grafts in periodontology and implant dentistry. In a series of experiments, we have investigated the adsorption and release of growth factors from four 3D collagen-based matrices and the migratory, proliferative, wound healing, and differentiation potential of primary human oral fibroblasts (hOF), primary human periodontal ligament cells (hPDL) as well as two osteoprogenitor cell lines in response to four clinically used matrices. The data show that the tested matrices can all be efficiently loaded with enamel matrix derivative (EMD) or recombinant growth factors such as TGF-β1, FGF-2, PDGF-BB, GDF-5, or BMP-2 and exhibit sustained growth factor release over a 13 day-period. All tested matrices significantly enhanced the ability of the investigated cell types to migrate towards the matrices as well as to efficiently repopulate an artificially generated wound gap covered by the matrices. Especially a novel Hydrated Dermal Matrix (HADM) triggered a strong migratory response and demonstrated significantly increased proliferative rates. The expression of genes encoding the angiogenic factors FGF-2 and VEGF-A was dramatically increased in cells grown on HADM, thus suggesting a good basis for accelerated vascularization. Additionally, the expression of major osteogenic markers was significantly increased in pre-osteoblasts grown on HADM, while the pre-coating of the 3D biomaterials with EMD or BMP-2 significantly boosted the osteogenic differentiation of the osteoprogenitor lines. Altogether, our results support favorable influence of the investigated 3D matrices on the recruitment and growth of cell types implicated in both oral soft and hard tissue regeneration. Among the evaluated matrices, HADM has consistently exhibited stronger positive effects on the oral cellular behavior. Furthermore, our data strongly support the biofunctionalization of the 3D matrices with EMD or BMP-2 as a potential treatment modality for soft tissue and bone defects in the clinical setting.

Q025

Clinical Performance of Immediately Placed Progressive-Line Implants: Results of a Prospective Clinical Study.

Georgia Trimpou

Polyclinic for Dental Surgery and Implantology, Department of Medicine, Goethe University, Frankfurt, Germany

Objectives: To assess implant success and survival on the intermediate (6 months) and long-term (up to 5 years) following immediate placement and immediate restored progressive type implants in the esthetic zone (14 to 24 FDI).

Material & Methods: A total of n=21 patients (21 implants) in need of extraction of a single tooth and implant treatment had received an immediate placement of a tapered, two-part implant with a progressive thread design (PL) for a single tooth replacement in the anterior maxilla. An immediate ‘non full-functional loaded’ temporal restoration was provided at the same day on a final patient-specific abutment (one abutment-one time concept). The final restoration was provided at 12 weeks (baseline). Implant survival and success (e.g. bleeding on probing - BOP, probing pocket depth – PD, mucosal recession – MR, pink esthetic score - PES) and patient-reported outcomes (PROM’S) were recorded at 6 and 12 months.

Results: An adequate primary implant stability (i.e. insertion torque >35 Ncm) was obtained at all but one sites. At 12 months, implant survival (n=20 patients) amounted to 100%. Non-significant changes to baseline were noted for mean BOP (2.5±28.2%), PD (-0.26±0.73 mm), and MR (0.0±0.4 mm) values. PES values amounted to 12.9±1.14 and 13.2±0.84 at 6 and 12 months. Patients expressed an overall high satisfaction both for the procedure and functional aspects.

Conclusions: The presented therapeutical protocol was associated with high survival and success rates and high acceptance by the patients on the short-term.
Q026

Pulp Preservation Therapies: From the lab to the Clinic and Back Again.

Ikhlas El-Karim
Queen's University Belfast, Belfast, Ireland

Advances in pulp biology had led to promotion of minimally invasive biologically based pulp preservation therapies, with many clinical studies showing early evidence of success. However, success of such therapies is undermined by the lack of appropriate tools for the diagnosis of pulp conditions. This lecture will exam scientific rationale of vital pulp treatments and the limitations of our current diagnostic system while highlighting the need for more translation and clinical research for evidence based application of pulp preservation therapies to clinical practice.

Q027

Next-Generation Targets: the Critical Role of MMP13 in Regulating Tooth Development and Reactionary Dentinogenesis Through Wnt Signaling.

Hal Duncan
Dublin Dental University Hospital, Trinity College Dublin, Dublin, Dublin, Ireland

The promotion of minimally invasive endodontic treatment has increased focus on pulp preservation techniques designed to avoid root canal treatment. However, there remain significant gaps in our knowledge regarding the molecular mechanisms and key mediators that regulate development, tertiary dentinogenesis and inflammation. This knowledge is critical when considering novel therapies to promote pulp healing.

We have recently reported that the collagenase Matrix Metalloproteinase-13 (MMP13) is highly expressed in odontoblasts and pulp tissue as well as human dental pulp cells. Using global MMP13-deficient mice, Mmp13−/− molars display critical alterations in dentine-phenotype, affecting dentine-tubule regularity, the odontoblast palisade, while reducing dentine volume and density. Furthermore, odontoblast differentiation-markers (Nestin, DSP) and Wnt-responsive gene Axin2 reduced in expression after MMP13-loss, with RNAseq analysis identifying downregulation of Wnt-signalling pathway in MMP13KO pulp tissue. Further data in MMP13KO a range of injury models has demonstrated that tertiary dentine is also reduced as is the expression of mineralisation, inflammatory and other key markers in KO samples.

The aim of this lecture is to summarise experimentation carried on MMP13 in developmental, repair and inflammatory models of the dentine-pulp complex.

Q028

Clinical Studies in Cariology – Is Big Data Better?

Martina Hayes
University of Cork, Cork, Ireland

The global prevalence of dental caries has decreased. Large population studies show a massive reduction over the past forty years in the prevalence of carious lesions among young children, adolescents, and adults. This decline can most likely be attributed to improved biofilm control, reduced sugar intake, increased use of fluoride, particularly in toothpaste, and an increase in regular dental attendance.

However, prevalence of this preventable disease remains high. Multiple systematic reviews have demonstrated that untreated cavitated dentine carious lesions make up the single most common disease that affects humans worldwide.

This lecture discusses the large datasets available to study trends in Cariology and whether or not there is a role for smaller clinical studies in this domain now that technology has facilitated the collation of “Big Data”.

Q029
Irreversible Pulpitis Treatment Rational: Pulp Regeneration and Antibacterial Potentials.
Imad About
Aix-Marseille University, Marseille, France
Pulp response to tooth traumatic injury/caries is highly dependent on the pulp inflammation degree due to its location within rigid dentinal walls. This explains why a rapid resolution of inflammation would favor the regenerative process which is key for a successful clinical outcome. Recent data have shown that injured pulp fibroblasts, which occurs under carious/trauumatic injury, provide a series of biologically active molecules via Complement system activation. Among these molecules, CSb and CSb-9 are involved in the local control of infection by killing cariogenic bacteria directly and inducing their elimination by phagoytosis. Other molecules such as C3a and C5a have shown a clear implication in pulp stem cell activation, proliferation, and recruitment. In addition, application of tricalcium silicate-based materials directly onto the injured pulp has been shown to modulate the inflammatory activity by decreasing pro-inflammatory growth factors and inflammatory cell recruitment. At the same time, the materials’ hydration/by-products release and their interaction with the soft tissues induce stem cell proliferation and recruitment. These data clearly demonstrate that fibroblasts withstand injury and that injured fibroblasts exert a local control of the initial steps of inflammation and regeneration. Overall, this presentation will explain how a good knowledge of bioactive tricalcium silicates properties combined with the pulp anti-inflammatory/regeneration potentials can provide a rational in symptomatic carious exposure treatment as well as pulp exposure.

Q030
Irreversible Pulpitis Treatment in Immature Teeth.
Papimon Chompu-Inwai
Chiang Mai University, Chiang Mai, Thailand
Tricalcium silicate cements increased the success of vital pulp therapy (VPT) and played an essential part for it in becoming a proposed definitive alternative for both immature and mature teeth in both children and adults. Moreover, several clinical studies reported on the success in using of tricalcium silicate materials in VPT procedures in teeth with clinical diagnosis of irreversible pulpitis. However, prior to the decision to perform any treatment for irreversible pulpitis teeth in children, clinicians should always take into consideration some unique characteristics of young permanent teeth in young patients (i.e., restorability of the affected tooth and stage of developing dentition, incomplete root formation and thin dentin wall, young bone, cost-effectiveness, etc.). Understanding these unique characteristics can lead to appropriate diagnosis and treatment planning of the tooth, when combine this consideration with the appropriate direct evaluation of the pulp and the use of tricalcium silicate cements will enhance the outcome of VPT performed in children.

Q031
Is Irreversible Pulpitis Treatment Possible in Mature Teeth?
Nessrin Taha
Faculty of Dentistry, Jordan University of Science and Technology, Jordan, Jordan
Irreversible pulpitis is defined as a state of vital pulp that is incapable of healing based on subjective and objective findings and thus traditionally root canal treatment was the default treatment of choice for carious pulp exposures, particularly in mature permanent teeth. However, histological studies have shown that while the clinical diagnosis coincide in the majority of the cases when evaluated histologically, the pulpal infection may be limited to the coronal portion of the pulp and therefore pulpectomy may not be necessary. The current approach of managing irreversible pulpitis has been reconsidered as a result of growing evidence from clinical trials reporting favourable outcome of vital pulp therapy (VPT), which in turn has led to new position statements published by professional societies on the treatment of teeth with carious pulp exposure and diagnosed with irreversible pulpitis. Clinicians often face the dilemma of which procedure and which material to use in VPT, and whether any material is considered superior in terms of outcome. This presentation will review available data on the clinical and histological diagnosis of irreversible pulpitis and discuss recent evidence in support of VPT in mature permanent teeth. Covering treatment considerations, selection of procedures, characteristics of currently available hydraulic calcium silicate-based materials with clinical case reviews.
Q032

Guided Biofilm Therapy & Dental Caries.

Hervé Tassery

Dental School, Aix-Marseille University, Marseille, France

Minimal intervention dentistry is now accepted for caries management. How and when to intervene using non-invasive, micro-invasive and invasive techniques according to the patient’s individual caries risk and the caries activity are the main factor. First, at each treatment steps: Non-Invasive, Micro-Invasive, or Invasive, it raised questions about how to clean without damaging, how to increase the diagnosis performance, and when to promote the use of ions released biomaterials subject to easily remove the biofilm. Second, the new diagnostic technologies combining images, magnification, and photonic signals like fluorescence, Infrared completely modified the paradigms of the Minimally invasive dentistry and caries diagnosis when combined with the GBT concept. The presence of surface cavitation is the starting point for micro-invasive restoration, caries activity is a warning sign to reverse or to moderate the caries process thanks to ions released biomaterials and cleansability is a moderating factor. The objective learning of this lecture will discuss about the GBT concept combined with new diagnosis tools and restorative paradigms.

Q033

Effect of Airflowing Powder-Erythirol on Dental Materials.

Behrouz Arefnia

Univ. Klinik für Zahnmedizin und Mundgesundheit, University of Graz, Graz, Austria

Biofilm removal on tooth surfaces is always accompanied by a loss of substance in the areas to be cleaned. We suppose that ideally the roughness of enamel surfaces is as smooth as possible after cleaning procedures without any significant substance loss. Subgingival instrumentation should only address the layer of endotoxin invasion from the root cementum. Over-instrumentation can lead quickly to the complete loss of the cementum and the resulting defect healing with epithelial cells. Also dental restorative materials are sourced for biofilm deposition and should be cleaned effectively without altering the characteristics of the material itself. The development of low abrasive new powders based on glycine, erythritol and trehalose is a renaissance for air polishing devices. A systematic review with 17 publications confirms these results also in the clinical application. Erythritol+chlorhexidine powder is also found to have perfect cleaning without defects in the enamel. The necessity of additional polishing is still under discussion. The aim of the lecture is to show how the different methods of surface treatment on enamel and root cementum and if non-abrasive powders have an effect on dental restorative materials in terms of surface alterations.

Q034

Airflowing Technology: What is it and how is it Different?

Marcel Donnet

Research Department, EMS, Nyon, Switzerland

How can you improve your prophylaxis procedure? How can you use at best the AIRFLOW system to remove optimally the biofilm? Come with me and visit the fabulous micro-world of powders to better understand what you have in your hand during the treatment. You will be transported at the micron scale along the powder pathway until it reaches the teeth. Afterwards, you will understand the importance of the used powder, that there are huge differences between powders, and that AIRFLOWING differs from AIRPOLISHING. It should become also easier to improve your technic for optimal treatment with maximum patient comfort.
Q035

**Can Guided Biofilm Therapy Alter the Oral Microbiome in a Short and Long Term Basis?**

**Victoria Sampson**  
*Private Practice, London, United Kingdom*

Periodontal disease has been considered one of the most significant global burdens of oral disease by the World Health Organisation. It is defined as a chronic, inflammatory disease of the periodontium and teeth-surrounding structures, causing irreversible damage and reduction in quality of life. The cause of periodontal disease is multifaceted and relies on local and systemic factors, one being oral microbiome dysbiosis. When levels of certain red pathogenic species are elevated in the mouth, it is believed this predisposes a patient to increased risk of periodontal disease. If these pathogenic red species are mechanically and chemically removed from the oral microbiome, periodontal disease symptoms should also significantly improve. Whilst a regular hygiene aims to remove plaque retentive factors and the biofilm from the mouth, it does not alter the biofilm and within hours a new biofilm (with the same bacteria) will re-establish if the underlying cause is not addressed. Guided Biofilm Therapy uses an antibacterial agent, erythritol, to allow for effective biofilm disruption. The agent is antimicrobial, anticariogenic and is thought to reduce formation of new pathogenic biofilms due to its anti-adherence property. Therefore, we suspect that regular GBT can positively influence the oral microbiome and allow for the recolonisation of commensal bacteria. Our hypothesis is that with regular guided biofilm therapy, pathogenic red complex species will significantly be reduced in short term and long term, allowing for oral microbiome symbiosis and therefore better prognosis of periodontal patients.

Q036

**Diagnosis and Early Management of Erosive Tooth Wear in Children and Adolescents.**

**Adrian Lussi**  
*University of Bern, Switzerland, Bern, Switzerland*

Dental erosion and specially erosive tooth wear are becoming increasingly important when considering the long-term health of the dentition. The clinical appearance is the most important sign for dental professionals to diagnose it.

This is of particular importance in the early stages of erosive tooth wear. The appearance of smooth silky-glazed sometimes dull enamel, intact enamel along the gingival margin and grooving on occlusal surfaces are some typical signs of dental erosion.

Adequate early management are very important and can only be initiated when the risk factors are known and interactions between them are present. Besides diet advise, modification of erosive food and beverages as well as modification of the acquired pellicle may enhance its protective quality and prevent dissolution of the dental hard tissues.

Recommendations for patients at risk for erosive tooth wear as well as early management will be discussed.
**Q037**

**Eating Disorders: how to Decipher the Clinical Complexity, as Well as in Psychological, Medical and Dental Consequences.**

Pierre Colon  
*University of Paris, Paris, France*

The management of carious lesions and of non carious lesions required before restorative treatments the control of etiological factors. Sometimes, while identification of these factors remain easy, their control is more critical. Eating disorders are now well known as critical situation with the higher level of suicide of psychiatric disorders. Somatic consequences can be severe with a very low BMI, osteoporosis, bradycardia due to potassium losses during vomiting. Dental complications observed are usually severe loss of dental tissues associated with TMJ disorders and a high level of aesthetic requirement. However, the identification of all etiological factors and their control is a difficult challenge. Nevertheless, the dentist is the resource person in the medical team. Through the aesthetic project, it’s possible for the patient to be part of his treatment and to recover some self-esteem. A good knowledge of eating disorders through 20 years of clinical practice in the field lead to advise youngsters clinicians in the management of complex clinical situations. How to identify all aetiological factors? how to determine their interaction? how to manage the relationship with the patient? How to build the treatment planning and how to slide slowly in accordance with the medical situation? The approach of “patient centered care”, well known in medicine but still new in dentistry could be the way to answer to these questions. Oral manifestations of eating disorders are rich of information regarding the habits of the patients. They are probably a new way to involve the dentist not only in the oral rehabilitation but also as a key person to be part of the treatment of the disease.

**Q038**

**Periodontal Health in Patients With Anorexia Nervosa and Bulimia Nervosa.**

Hélène Rangé  
*Department of Periodontology, Université Paris Cité, Paris, France*

People with eating disorders suffer from a mental disorder that negatively affects their mental, social and physical health. The three most frequent eating disorders are binge eating disorder, bulimia nervosa, and anorexia nervosa. There is significant overlap between anorexia nervosa and bulimia nervosa, notably regarding the purging behaviors (to avoid weight gain): vomiting, drugs’ misuse, and excessive exercise. Both conditions have negative effects on the oral tissues, their symptoms can range from slight to severe and oral health professionals are often among the first to suspect an eating disorder. However, until recently studies have reported conflicting results regarding the oral hygiene and periodontal health conditions. On the one hand, the eating disorder sufferer exhibits personality traits supposed to lead to overzealous toothbrushing. On the other hand, they suffer from depressive comorbidity with low interest in oral hygiene practices. The aim of this conference is to discuss the emerging evidence on the periodontal health conditions in patients with anorexia nervosa or bulimia nervosa, notably plaque-induced gingivitis and gingival recessions. In addition, the risks and efficacy of periodontal treatment including periodontal plastic surgeries in people with an eating disorder will be highlighted.
Q039  
**Autografts: Osteogenicity and Other Properties.**  
Reinhard Gruber  
University of Vienna, Vienna, Austria  

Autologous bone is a favorable graft because of its osteoconductive properties, osteogenic activity, and being a source of growth factors. Its limited amount and the morbidity upon harvesting, however, has caused the search for alternative sources including allografts. Allografts are more passive in their capacity to support bone regeneration. Moreover, alternative sources of autografts have been proposed, namely dentin. Based on these claims, the first aim will be to briefly recapitulate the history of autograft and allograft research based on knowledge we gained from preclinical models. Second aim is to share our data on bone and dentine lysates by a combined proteomics and RNA sequencing approach. We have identified acid bone and dentine lysate to be a rich source of TGF-β, hold an anti-inflammatory activity, and potentially modulate bone regeneration in rat calvarial defects. Third and final aim is to show that allografts maintain their TGF-β activity. This presentation should be considered a primer for future research with the overall goal to understand the favourable properties of autografts at a molecular and cellular level, and how we can implement this knowledge to improve the clinical performance of allografts and other bone substitutes.

Q040  
**Regeneration of Cranio-Maxillofacial Bone With Autologous Adipose Tissue Derived Stem Cells and Biomaterials.**  
Susanna Miettinen  
Adult Stem Cell Group, Faculty of Medicine and Health Technology, Tampere University, Finland  
Research, Development and Innovation Centre, Tampere University Hospital, Tampere, Finland  

Insufficient vascularization is a major obstacle for clinical application of tissue engineered transplants including bone. The ambition is to provide an environment rich in vascular networks to achieve efficient osseointegration and accelerate functional restoration after implantation. Of particular interest is the microvasculature that is crucial for oxygen and nutrient delivery. Microvascular networks in 3D can be formed in vitro through the coculture of endothelial cells (ECs) with supporting pericytic cells. Mesenchymal stem/stromal cells (MSCs) derived from bone marrow (BMSCs) and adipose tissue (ASCs) are an attractive choice for pericytes due to their natural perivascular localization and ability to support formation of mature and stable microvessels. Furthermore, they are most used cell types for bone tissue engineering and clinical trials focusing on bone regeneration. Here, our aim was to explore the vasculogenic potential of human ASCs and BMSCs in a perfusable microfluidic device. BMSCs and ASCs were co-cultured with ECs in a fibrin hydrogel in a microfluidic chip. We compared the capacity of BMSCs and ASCs to induce the formation of mature microvascular networks by ECs and to differentiate into pericytes. We studied the effect of MSCs on vessel characteristics such as area, diameter, length, and perfusability. Interstitial flow across the hydrogel area was measured daily in EC-BMSC and EC-ASC cocultures using fluorescence imaging. We assessed MSCs pericytic differentiation in terms of pericyte area and pericyte coverage by immunohistochemical staining and quantitative analysis. Furthermore, we evaluated the expression of main vasculogenesis related genes.

We demonstrated that using MSCs of different origin resulted in vascular networks with distinct phenotypes. Both types of MSCs supported formation of mature and interconnected microvascular networks. However, BMSCs induced formation of fully perfusable microvasculature with larger vessel area and vessel length compared to ASCs. Co-culture with ASCs resulted in only partially perfusable microvascular networks. Immunostainings revealed that BMSCs had greater potential to differentiate towards pericytes than ASCs. The gene expression analysis revealed significant differences in the expression of endothelial-specific and pericyte-specific genes, as well as genes involved in vasculature maturation and remodeling. Overall, our study provides valuable knowledge on the properties of BMSCs and ASCs as vasculature supporting cells and highlights their distinct directing role in the regulation of microvascular phenotype that might have implications in bone tissue engineering applications.
Q041

**Bone Marrow Mesenchymal Stem Cells in Reconstruction of Alveolar Bone – RCT Clinical Trial.**

Gudveig Cecilie Gjerde Gjengedal, Kamal Mustafa

*University of Bergen, Bergen, Norway*

**Introduction:** In a non-controlled recent clinical study, autologous bone marrow derived stem cells combined with biomaterial induced new bone formation. This has been reported as promising new approach for reconstruction of atrophied posterior alveolar mandibular ridges. We aimed in the present study to demonstrate the efficacy of this therapeutic ATMP approach in a randomized multicenter controlled clinical trial.

Our research group has pioneered the field of bone tissue engineering and demonstrated feasibility, safety, and efficacy of the combination of Biphasic Calcium Phosphate (BCP) granules and autologous bone marrow derived stem cells (MSC) in preclinical studies and early phase human clinical trial (n= 11 patients). Successful regeneration of the alveolar bone in the pilot trial was evident in radiographic and histological findings [1]. The findings were supported by the ability of the newly formed tissue to accommodate a dental implant and withstand the forces of mastication on daily bases. Therefore, the present work is aimed to perform phase IIb multicenter randomized controlled clinical trial for regeneration of mandibular bones of patients prior to dental implants using autologous MSC.

**Methodology:** Patients with a need for bone reconstruction of residual edentulous ridges in both the mandible and maxilla due to bone defects with a vertical loss of alveolar bone volume and/or knife edge ridges (≤ than 4,5 mm) unable to provide adequate primary stabilization for dental implants were included in the clinical study. Autologous bone marrow MSC were expanded, loaded on BCP (MBCP+™; Biomatlante, France) and used to augment the alveolar ridges. After five months bone biopsies were harvested at the implant position site and implants were installed in the regenerated bone. The implants were loaded after 8 -12 weeks. Safety, efficacy, quality of life and success/survival were assessed. Six clinical centers, 5 different countries participated. Bone grafts harvested from the ramus of the mandibles were used as control in the study. Results: 57 patients have so far been screened and enrolled in the study. 28 patients have been treated in the test group, 9 in the control group and 5 withdraw before treatment.

**Conclusions:** The results this far indicates that the use of bone marrow derived stem cells in the applied protocol for augmentation of the atrophied mandibular ridge have results comparable to the gold standard; autologous bone transplantation with predictable longtime results.

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Q042

**The art & Science of Minimally Invasive Operative Caries Management.**

Avijit Banerjee

*King’s College, London, United Kingdom*

In his introductory presentation as part of the symposium with the same title, Prof Banerjee will introduce and outline the clinical domain of operative minimally invasive dentistry (MID) as part of the overall holistic, patient-focused, oral healthcare team-delivered minimum intervention oral care (MIOC) delivery of contemporary dental caries management. He will focus on the evidence-based management of deeper cavitated carious lesions, the selective caries removal concept and linking carious dentine histology with excavation technologies and the contemporary bio-interactive restorative materials used to restore such cavities.
Q043
The Science of bio-Interactive Adhesion.
Salvatore Sauro
Dental materials & Minimally Invasive Dentistry, University CEU Cardenal Herrera, Alfara del Patriarca, Spain, Spain
In 2022, the hybrid layer turns 40 years old. Indeed, Nakabayashi et al. (1982) published the first article about the hybrid-layer formation theory, although Kramer and McLean in 1952 already observed a resin-penetrated zone within the resin-dentine interface created by the glycerolphosphoric acid dimethacrylate. This latter monomer was a revolutionary idea of Oscar Hagger (in 1949), who introduced it within the formulation of the Sevriton Cavity Seal; he had in fact invented a first sort of self-etching adhesive. Nowadays, most of the direct and indirect bonding procedures rely on the chemical bonding properties of functional monomers present in modern resin-based materials. However, even though dental adhesive systems have improved considerably over the last twenty years, several issues regarding premature reductions in bond strength, interface and marginal degradation are still a matter of concern. This lecture will attempt to bring together a number of demonstrations to show the current state of the art on the bonding durability. Moreover, Innovative new approaches to remineralise the resin-dentine interface may protect hybrid layers from different types of degradations over time, and have a therapeutic role in caries prevention.

Q044
The art of bio-Interactive Material Clinical Applications.
L. Sebnem Turkun
Restorative Dentistry, Ege University, Izmir, Turkey
Minimally invasive operative dentistry has nowadays a clear place in daily primary care dental practice, with practitioners and patients being aware of its importance and benefits. Minimal invasive restorative operative techniques include selective caries removal, adhesive biomaterials with bio-active/interactive properties and biomimetic restorations. Advances in adhesive dentistry, including newly developed bio-active/interactive materials are now expected to induce repair of the residual affected demineralised dentine, resulting in a significant impact on restoration longevity and its clinical performance. In this presentation, an introduction to bio-active/interactive materials followed by the art of their clinical applications will be addressed in the light of some clinical cases.

Q045
The Science of Modern Composite Techniques.
Pekka Vallittu
University of Turku, Turku, Finland
Development of dental and medical biomaterials has been limited to use only isotropic “bulk-like” materials until two decades ago, when the first clinically applicable fiber-reinforced composites (FRC) have become to the clinical use. Present applications of FRC can be found in all disciplines of clinical dentistry and in certain applications of bone reconstructive surgery. All of these applications are having fibers of glasses of various compositions in the resin-based matrix of FRC. Orientation of fibers in the polymer matrix may vary from unidirectional to bidirectional weaves and random fiber-oriented mats. On the other hand, fibers can also vary in terms of length. Continuous and discontinuous (short) fibers are both utilized in dentistry nowadays. Discontinuous fibers in a reinforcing purpose are having aspect ratio which is higher than 20. Incorporation of fibers of this kind to the FRC increases mechanical properties of the composite, especially clinically important fracture toughness is considerably increased. Applications for composites of these kinds are in fixed dental prostheses, periodontal splints, retainers and fillings. Other properties of FRC which relate to the direction of fibers, like optical properties and bonding properties are also having their implications in dentistry, like in root canal posts, where transmission of curing light is a desired property for polymerization of an individually formed fiber post and the luting cement. Successful use of FRC in clinical dentistry requires comprehensive understanding of the mechanism of action of the components of FRCs and the loading conditions where the device will be used. Lecture will show aspects of the theory behind function of FRC and what are future perspectives for FRCs in dentistry and bioactive surgical FRC implants.
Biological Aspects of Modern Composite Materials.
Jan T. Samuelsen
NIOM, Oslo, Norway
Safety for dental personnel and the dental patient is an essential requirement for dental materials. Knowledge of potential interactions between dental materials and living cells makes a good foundation for their safe use. The primary source for such knowledge is in vitro studies that point out factors affecting exposure conditions and how these exposures influence living cells.

The composition of modern composite materials is complex. Although the materials cure well, they are not inert, and dental patients and dental personnel are exposed to their constituents. Several studies have searched for knowledge on how these constituents affect human cells, oral bacteria, and the host-microbe interaction. In addition to summarizing the current state of the art, this lecture will give attention to how the in vitro research strategies are changing and how new laboratory techniques gain a more accurate understanding of the biological aspects of the materials. More detailed knowledge of the biological interactions subsequently makes it possible to develop standardized in vitro tests that predict the potential of specific clinical side effects from the materials more correctly than the currently used tests.

A more accurate understanding of the biological aspects of dental composite materials and better tests to reveal possible clinical issues are elements that help the development and safer use of the materials.

Clinical Aspects of Modern Composite Techniques.
N. J. M. Opdam
Dentistry, Radboud University Nijmegen, Nijmegen, Netherlands
The clinical performance of composite restorations may be dependent from many variables such as dentist factors, patient factors, material aspects and technical aspects related to the procedure. In this lecture an overview of available evidence of risk factors related to the survival of composite restorations is presented with the focus on technical aspects related to the clinical procedure to place a composite resin restoration.

Antibiotic Exposure: the Growing Concern About off-Target Effects.
Fernanda Petersen
University of Oslo, Oslo, Norway
Antibiotic prescription to humans has been largely based on a «better safe than sorry» approach, with little attention paid to unwanted effects. In the awake of the antibiotic crisis and the increased focus on a balanced microbiome for health, our attention is now turning to risks, including the unwanted collateral effects on the microbiome. Although antibiotics are used to target specific bacteria at infection sites, off-target effects are virtually unavoidable. Antibiotic use exposes the microbiome to different antibiotic concentrations. These are usually lower than in the targeted infection sites, but can nevertheless disturb the microbiome and select for and promote antibiotic resistance. Recent advancements in the field of metagenomics are already revealing the impact of antibiotics in enriching for antibiotic resistance among commensals and major pathogens at off-target sites. This knowledge is of relevance to understand not only the dynamics of antibiotic resistance, but also the impact of interventions, including improvement in prescription practices.
Q049
Bioinformatics Tools and Databases for Studying Resistome.
Tsute (. Chen
Harvard School of Dental Medicine, Boston, Massachusetts, United States
Antimicrobial resistance (AMR) is a major public health threat and is rising to dangerously high levels worldwide as new resistance mechanisms are emerging and spreading globally. Antibiotics is becoming less effective and it has grown increasingly difficult, even impossible in some cases, to treat common infectious diseases such as pneumonia. Although the situation looks bleak, researchers are using innovative ways to combat this global public health threat. Bioinformatics tools and databases are being recruited to expedite diagnostic, identification, and characterization of AMR, as well as discovery of novel antibiotics and therapeutics. AMR is mainly mediated by the antibiotic resistance genes (ARGs) but the resistance to antibiotics spans all known classes of natural and synthetic compounds. This is because bacteria in the environment encounter a myriad of antibiotics and are constantly evolving with varies corresponding sensing and evading strategies. The antibiotic resistome encompasses all types of resistance causing ARGs and elements such as acquired, intrinsic, silent/cryptic, proto-resistances. Thus, efficient methods to identify and characterize the resistome will enhance AMR mechanism discovery and diagnostics. Traditionally AMR is identified by in vitro laboratory assays such as susceptibility testing and molecular detections. Increasingly, whole-genome shotgun sequencing (WGS) has been used to detect ARGs in silico, enabling efficient prediction of resistance phenotypes and discovery of novel resistance variants. The in silico approach requires comprehensive ARG databases and robust software tools. There are many databases and tools using a variety of approaches and data sources. This presentation will review currently available tools and databases for studying AMR and resistome.

Q050
Bacterial Warfare Involving Antimicrobial Factors Produced by Oral Streptococci
Michael J. Federle¹, Britta E. Rued²
¹University of Illinois, Chicago, Illinois, United States, ²Chicago, University of Illinois, Chicago, Illinois, United States
Streptococcus mutans, an etiological agent of dental caries, is proficient in bacterial cell-to-cell communication. Communication networks, colloquially termed quorum sensing (QS) systems, coordinate gene expression across bacterial communities and are known to govern many behaviors including biofilm development, horizontal gene transfer (natural competence), and production of antimicrobial factors. In addition to QS systems that regulate competence (ComRS) and bacteriocin production (BlpRH), we characterized the PdrA/Wgk system that regulates the production of a ribosomally synthesized and post-translationally modified peptide (RiPP) termed tryglysin that has antimicrobial properties. PdrA and its corresponding short, hydrophobic pheromone (SHP) are members of the Rgg family of QS systems that are prevalent among species of Streptococcus. We find that synthetic SHP pheromone is sufficient to induce expression of the wgk biosynthetic operon, which utilizes a radical-SAM enzyme to generate tryglysin. The mechanism by which tryglysin inhibits growth of other gram-positive bacteria remains unclear, but we find that biofilm morphology and development of an S. mutans pdrA mutant treated with tryglysin is dramatically altered from wild type. This suggests that PdrA is involved in S. mutans’ response to tryglysin and that tryglysin impacts biofilm development. As QS systems are susceptible to inhibition by small molecules, the results are significant as they may provide a means to alter S. mutans biofilm formation and therefore caries potential.
Q051
Global Challenges in Dental Education on Antimicrobial Resistance.
Roger Junges
University of Oslo, Oslo, Norway
The antimicrobial resistance crisis is one of the biggest threats to global health, food security, and societal development. Even though the development of antimicrobial resistance is a natural process in microbes that has been happening since ancient times, the overuse and misuse of antibiotics in health care is one of the main drivers in propelling the rapid development of the resistance crisis. In oral health care, antibiotic prescriptions are part of the daily routine and dentists are responsible for 5-10% of all antibiotic prescriptions in primary care. While in many cases the use is indeed necessary, recent evidence has indicated that there are a significant number of unnecessary prescriptions by dentists as well. Further, there is limited data regarding awareness about antibiotic resistance and prescription practices in many world regions, and the seriousness of the issue requires a coordinated global approach. As the control and prevention of infections remains one of the cornerstones of a healthy global population, there is a need to revisit the topic of antibiotic resistance on dental curricula. It is timely to identify challenges faced by stakeholders and reveal opportunities for collaboration across institutions and sectors. As we move forward, we need to ensure that antibiotics are still effective and that antibiotic stewardship initiatives are an integral component of dental education programs.

Q052
Pathogenic Mechanisms Underlying Smell and Taste Disturbances With Focus on COVID-19
Alexander Fjældstad1,2
1Flavour Institute, Aarhus University, Aarhus, Denmark, 2Department of Otorhinolaryngology, Flavour Clinic, Gødstrup, Denmark
Disturbances of the sense of taste and especially the sense of smell are common symptoms affecting up to 20% of the population. Although this prevalence has been known for years, the COVID-pandemic has shed an unprecedented focus on these symptoms both in the general population, among health care professionals and researchers. The pandemic has advanced our knowledge of post infectious chemosensory disturbances and emphasized the need to address these debilitating symptoms in patients. By addressing the heterogenic etiologies of these disorders this talk will focus on describing the numerous different pathogenetic mechanisms of taste and smell disturbances, with a particular focus on COVID-19.
Long COVID-19 and Olfactory Disorders

Therese Ovesen1,2

1ENT Department, University Clinic for Flavour, Balance and Sleep, Herning, Denmark, 2Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

Smell and taste disorders have been reported in up to 70-85% of patients with COVID-19 infections. The majority of cases resolve spontaneously during the first three months. However, 5-10% of all COVID-19 patients develop long COVID-19 syndrome with several symptoms of which smell and taste disorders are some of the most prevalent. Before the COVID-19 era, post-infectious smell and taste disorders were also frequent. The current question is whether long COVID-19 differs from non-COVID-19 post-infectious smell and taste disorders, which is of interest in terms of prognostication. Therefore, we present a comparative study of long COVID-19 and non-COVID-19 post-infectious smell and taste disorders.

Patients were recruited from the Flavour Clinic, University Clinic for Flavour, Balance and Sleep, Ear-nose-throat Department, Gødstrup Hospital, Denmark. Post-COVID-19 patients were divided into two groups: simple, i.e. smell and taste disorders as the only long-COVID-19 complaint; and complex, smell and taste disorders along with other serious long-term sequelae after COVID-19. Besides subjective questionnaires, a thorough ear-nose-throat examination, Minimal Mental State Examination (MMSE), orthonasal smell test (Sniffing’s sticks TDI scores), retro-nasal test and taste screening were performed.

Cases with long-COVID-19-related smell and taste disorders deviated significantly from non-COVID-19 post-infectious cases; the patients were younger, had a lower occurrence of anosmia/ageusia, more frequently had distorted senses (parosmia and phantosmia), and generally had higher TDI scores. Parosmia and phantosmia were more prevalent among patients with simple long-COVID-19 complaints than among complex cases and their quality of life scores were more affected by their distorted senses. However, the differences between COVID-19 and non-COVID-19 cases may exclusively be due to selection except with regard to sensory distortion. In terms of patient counselling, it is utmost important to obtain knowledge about the development of the sensory distortion with time as it may lead to anxiety and depression.


Preet B. Singh

Institute of Clinical Odontology, University of Oslo, Oslo, Norway

According to WHO, more than 5.27 billion people worldwide and 1.4 million people in Norway have been diagnosed with COVID-19, as of June 2022. While most patients develop mild or uncomplicated forms of COVID-19, it is estimated that approximately 14% are associated with severe acute respiratory infection and may require hospitalization and oxygen support, and 5% require admission to an intensive care unit. Sudden smell loss is a specific early symptom of COVID-19, with an estimated prevalence of 40% to 75% in a study conducted by the Global Consortium for Chemosensory Research. Whereas loss of smell after COVID-19 is broadly addressed, other oral disturbances like loss of taste function, oral dryness, burning mouth sensation, and risk of dental caries are less studied. In line with this, circumstantial evidence indicates that oral disturbances including, taste-, salivary-, and trigeminal functions are quite prevalent in COVID-19 patients, and this may affect their physical and mental health, as well as their dietary behavior. Thus, it is critical to understand the range and time course of oral disturbances in COVID-19 patients and why they occur. Long COVID is defined as a condition characterized by long-term sequelae appearing or persisting after the typical convalescence period of COVID-19 that continue for more than 12 weeks and are not explained by an alternative diagnosis. This lecture focuses on (i) what kind of problems Long COVID patients have with smell, taste, chemesthesis, and salivary function, (ii) how long after undergoing COVID-19 infection these problems occur, (iii) management regimes for treatment of these disorders, (iv) and how long does it take after COVID-19 infection before they regain normal smell, taste, trigeminal and salivary function, with or without treatment.
KEYNOTE LECTURES abstracts

Q001
Silver Agers, but Silver Gone: Are Demographics, Epidemiology and Dental Restorative Care Approaches in Sync?
Falk Schwendicke
Oral Diagnostics, Digital Health, Health Services Research, Charité - Universitätsmedizin Berlin, Berlin, Germany
Our society changes – and so does dentistry. We face an ageing group of individuals with growing, not decreasing dental needs – but increasing difficulty to provide care to them! In parallel, the dental material world is rapidly advancing, partially facilitated by the face down of dental amalgam as former standard restorative material. The planned keynote will outline these challenges and showcase that population demographics and dental epidemiology will need to be matched by dental care approaches and material answers.

Q055
Clinically-Inspired Translational Microbiology: From Standardisation to Molecular Mechanisms.
Cher Farrugia
Bristol Dental School, University of Bristol, Bristol, United Kingdom
Dr. Farrugia will be presenting research that led to the 2022 BSODR President’s Prize focusing on her contributions towards multidisciplinary translational microbiology. The oral cavity has the second largest and most diverse microbiota in the human body, some of which are associated with colonisation of dental devices and their subsequent failure. A wide range of methods are used to test antimicrobial properties, often using simple techniques with little mimicry of the clinical environment/application, making inter-paper comparisons and clinical translation challenging. Research investigating antimicrobial testing’s clinical applicability resulted in an international multidisciplinary publication with recommendations for antimicrobial testing of dental devices, some of which were successfully adapted in "ISO/DIS 3990 Dentistry-Evaluation of antibacterial activity", the first standard of its kind. Oral microorganisms have been shown to have effects beyond the oral cavity. Studies have shown an association between periodontal disease and increased cardiovascular disease risk: perio-cardio link. Despite this evidence, investigations of the molecular mechanisms involved are generally limited to 2D single species bacterial infections. Innovative in vitro and real-time zebrafish embryo in vivo models used to investigate the role of P. gingivalis, its Outer Membrane Vesicles (OMV) and multispecies infections’ role in the perio-cardio link will be presented in the second part of the presentation. This work deduced that gingipain in P. gingivalis cells, as well as independently through P. gingivalis OMV, damages endothelial cell adhesion molecules, which in turn leads to increased endothelial permeability. This pathologic molecular mechanism can therefore play a role in the initiation or potentiation of vascular damage. Later work highlighted how F. nucleatum induces vascular damage through this mechanism, as well as subspecies variations in pathogenicity in multispecies infections. In the zebrafish embryo realtime-model, multispecies infections of P. gingivalis, F. nucleatum and T. forsythia showed microbial aggregation, confirming the importance of multispecies assessments. The collection of work presented highlights the importance of clinically-guided research, as well as the significance of multidisciplinary innovations to aid in unravelling the complex role of the oral microbiota in our mouths and beyond.

Q002
Bioactive Dental Concept: When and how Using Ions Released Biomaterials
Hervé Tassery
Aix-Marseille University, Marseille, France
Minimal intervention dentistry is now accepted for caries management. How and when to intervene using non-invasive, micro-invasive and invasive techniques according to the patient’s individual caries risk and the caries activity are the main factor. First, at each treatment steps: Non-Invasive, Micro-Invasive, or Invasive, it raised questions about how to clean without damaging, how to disinfect the deep dentine layers, when to promote the use of ions released biomaterials. The presence of surface cavitation is the starting point for micro-invasive restoration, caries activity is a warning sign to reverse or to moderate the caries process thanks to ions released biomaterials and cleansability is a moderating factor. More in case of proximal lesions presence or not of bondable enamel in gingival margins completely modified the biomaterials choices. In case of initial, moderate or extensive occlusal cavitation, the clinical criteria include the individual caries susceptibility, the
caries lesion activity and lesions accessibility. The objective learning of this lecture will discuss the use of ions released biomaterials with an unconventional angle, in case of Initial, Moderate and Extensive carious lesions.

Q003
Oral Health Profile and Cardiovascular Risk: the Nutritional Pathway.
Philippe Bouchard
University of Paris, Par, France
Blood biomarkers of cardiovascular disease and clustering algorithms that identify the patient profile at risk for coronary heart disease support the detrimental role of impaired masticatory capacity in the association between poor oral health and cardiovascular risk. The synergistic link between the nutritional and inflammatory pathways may be the cornerstone of the relationship between periodontitis/dental caries, the two most prevalent oral diseases, and the incidence of cardiovascular events. The aim of this presentation is to explore the impact of masticatory capacity as a potential mediator in the relationship oral health-cardiovascular health.

Q004
Adhesion to Ceramic Surfaces: Does 10-MDP Primer Performs Better Incorporated or Separated From the Resin Cement?
Olivier Etienne
University of Strassbourg, Strassbourg, France
Objective. The objective of this study was to evaluate the bond stability between enamel and zirconia ceramic surface (Y-PSZ) enhanced with a glass-ceramic coating. Comparison of shear Bond strengths after 150 days of aging in water was done between the new modified surface and 2 classical surface treatments and lithium-disilicate-based ceramic, using two dual-cured adhesive resin cements (ARC).

Materials & Methods. 80 specimens composed of ceramic cylinders and enamel discs were obtained and 8 experimental groups out of them (n=10). Sixty cylinders of zirconia (Katana STML zirconia (Kuraray, Japan) were assigned to 3 groups according to the surface treatments: control, as milled/sintered surface (ZRCT); tribiochemical silica-coating (Cojet Sand, 3M ESPE, Seefeld, Germany) (ZRTC); glass-ceramic coating (IPS e.max Zirpress) (ZRZP). Twenty cylinders of lithium disilicate were used as milled/sintered surface (IPS e.max CAD (Ivoclar-Vivadent, Schaan, Liechtenstein) (ECAD). The cylinders of each group were further divided into 2 subgroups according to the resin cement as follows: Panavia F2.0 (-PF) and Panavia V5 (-PV). Specimens were stored in distilled water for 150 days before shear bond strength (SBS) tests. The mode of fracture was analyzed using digital microscope and data were statistically analyzed using two-way ANOVA and a post hoc Tukey test (p < 0.05).

Results. Group ECAD-PF recorded the highest SBS (31.75±2.2) and group ZRCT-PF the lowest (5.59±1.1). Two-way ANOVA showed that ceramic did have a statistically significant effect on SBS (F(3,72)=38.95, p<0.001) while ARC did not (F(1,72)=2.40, p=0.126). Tukey post hoc test revealed no statistical difference between the ZRZP group and the ZRTC and ECAD groups.

Conclusion. Within the limitations of this study, a long-term durable bond was achieved with the heat press ceramic coating. This new surface treatment could be recommended for RBFDPs for its fracture resistance and bonding ability.
**Objectives** The BRIGHT trial is investigating clinical and cost-effectiveness of a two-part complex behaviour change intervention for UK school pupils (11-14 years-old), to increase toothbrushing frequency and reduce caries experience:

1) A classroom-based lesson (CBL) provided by teachers in 42 secondary schools; and
2) SMS (text messages) sent to participants’ mobile phones.

Following the CBL, SMS messages were sent twice daily through secure data management services by University of Dundee’s Health Informatics Centre. Participants ended and restarted reminders by texting “STOP” and “START”. Messages were sent explaining the number was, however, not monitored and there would be no reply. Despite this, some participants still sent other replies. **Aim:** To analyse text message responses (other than STOP/START) and identify data trends.

**Methods** The East of Scotland Research Ethics Committee (17/ES/0096); https://www.fundingawards.nihr.ac.uk/award/15/166/08. Data were collated and analysed in Microsoft Excel.

**Results** Of 2,260 participants sent text messages, there were 8461 responses from 1388 participants (61%). Between one (n=360) and 585 (n=1) SMS text responses per participant were received (mean=6.1; median=3; mode=1) with 1289 “STOP” and 48 “START” messages, leaving 7,124 non-START/STOP responses. Three participants were responsible for 929 responses (585, 192, 152), all of which were affirmative; “OK”, “yes” etc. 59.6% of non-START/STOP responses (4252/7,124) indicated agreement to brush teeth or already brushed. There were almost three times as many “positive” messages (n=454) than “negative” messages (n=175) and 152 questions relating to toothbrushing or the trial process (1.7% of responses).

**Conclusions** Although there was no requirement to reply to the brushing reminder texts and no response sent back, young people in the trial felt compelled to affirm their intention or positive action. Most text responses (66%) were positive or indicating agreement that participants would brush/had brushed their teeth.

<table>
<thead>
<tr>
<th>Reply Theme</th>
<th>Count of Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indicating agreement (“done,” “I know,” “OK,” “yes”)</td>
<td>4252</td>
</tr>
<tr>
<td>Other (messages not relevant or nonsensical)</td>
<td>1485</td>
</tr>
<tr>
<td>Positive message (“thanks”, “hello”, “bye,” other)</td>
<td>454</td>
</tr>
<tr>
<td>Indicating disagreement (“no,” “forgot” etc.)</td>
<td>435</td>
</tr>
<tr>
<td>Negative (negative, expletive)</td>
<td>175</td>
</tr>
<tr>
<td>Questions (process queries, questions about brushing etc.)</td>
<td>152</td>
</tr>
<tr>
<td>Who is sending texts?</td>
<td>72</td>
</tr>
<tr>
<td>Change of phone number request</td>
<td>53</td>
</tr>
<tr>
<td>Change timing of messages request</td>
<td>46</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>7124</strong></td>
</tr>
</tbody>
</table>
O002

Optimising Value-Based Preventive Dental Care.

Christopher Bannister, Anwen Cope, Paul Harper, Anup Karki, Sarah Peddle, Brenda Walters, Ivor Chestnutt

Dental Public Health, Cardiff University, Cardiff, Wales, United Kingdom, Cardiff University, Cardiff, United Kingdom, Public Health Wales, Cardiff, United Kingdom, PPI representative, Cardiff, United Kingdom

Objectives In many dental care systems current clinical pathways reflect a “drill and fill” ethos, rather than a preventive approach. This study utilised operational research techniques to model skill-mix use in a preventive-led dental care system and compared this to current care delivery.

Methods Current clinical pathways were described through analysis of routinely-collected consultation data. Twenty-one preventive pathways were developed following an evidence-based clinical guideline synthesis and clinician and patient consensus events. Three models of skill-mix use were developed using UK General Dental Council (GDC) guidelines. Timings of 55 common clinical procedures were obtained via a cross-sectional study of Welsh dental professionals.

A demand and optimisation model with a web-based user interface was developed. The population used to demonstrate the model was based on 236,490 individuals (65,513 children, 179,977 adults) who visited NHS General Dental Service (GDS) practices in Wales between July 2018 and September 2019. The outputs of preventive pathway scenarios follow an intention-to-treat approach and assume 90% attendance rates.

Results Under preventive-led scenarios, the number of treatment items delivered increased 47.7% compared to current practice, principally driven by a 372% increase in preventive interventions, primarily for the highest-need patients. Using existing patterns of skill-mix use, preventive-led scenarios incurred 24.5% more staff hours than current care and 27.1% greater staff costs. However, whilst incorporating maximal skill-mix into preventive-led scenarios incurred 11.0% more staff hours than existing skill-mix patterns, total staff costs were 23.9% lower.

Under this final scenario, dental therapists and hygienists delivered a fifth of all care.

Conclusions This work suggest that it may be possible to deliver a preventive-led model of care within existing resources but only if skill-mix use is optimised. This challenges previous policy which has suggested that reducing the frequency of attendance of low-risk patients may alone free-up sufficient resource to reorientate care.

O003

EVALUATION of an ORAL CARE PROGRAMME

Annica Almståhl, Charlott Karlsson, Jessica Skoogh Andersson

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Objectives To evaluate the effect of an oral care programme for patients undergoing treatment for cancer in the head and neck region regarding mucositis.

Methods Patients scheduled to undergo curative treatment for head and neck cancer are included in 5 regions in Sweden. All patients are advised to clean their teeth twice a day and to avoid smoking and alcohol. The patients are randomized to an intervention group or to a control group. Patients in the intervention group rinse with a sodium chloride/bicarbonate solution 5 times/day and record their oral hygiene measures and rinses in a diary. They visit the dental hygienist once a week for professional oral care and get a reinstruction in oral hygiene if needed. Patients in the control group follow ordinary routines (visit the dental hygienist once a week for professional oral care). Mucositis is scored using the Oral mucositis assessment scale (OMAS) at baseline, weekly during radiation therapy and 1 and 3 months post treatment.

Results 62 dentate patients (48 men, 14 women), mean age 58 years have been included so far. 29 patients were randomized to the intervention group and 33 to the control group. The most common diagnoses in both groups were tonsil cancer and tongue base cancer (55% and 79%) and the most common treatment modality were RT+chemotherapy (45% intervention group and 61% control group). The mean scores for ulceration week 3-6 during RT was 1-1.9 points lower in the Intervention group compared with the Control group.

Conclusions A lower degree of ulceration during treatment might decrease oral pain which in turn is positive for the patient’s ability to eat, drink and perform oral hygiene.
Exploring Awareness and Attitudes Towards Sustainable Dentistry at Semmelweis University
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\textsuperscript{1}Department of General Dental Preclinical Practice, Semmelweis University, Budapest, Hungary, \textsuperscript{2}Semmelweis University Faculty of Dentistry, Budapest, Hungary

**Objectives**

The healthcare sector, including dentistry, has a significant ecological footprint, which need to be measured in order to develop mitigation strategies, for which the attitudes of stakeholders should be examined first.

**Objective:** Our objective was to map general awareness and attitudes towards sustainability in daily life and at work among a group of dental professionals, and to assess their willingness and readiness to change.

**Methods**

Method: A voluntary and anonymous, representative online survey with 66 questions was administered to dentists, dental students, dental assistants, dental technicians and students of the listed disciplines, regardless of age and gender, who were working or studying at the departments of Semmelweis University Faculty of Dentistry. Data collection took place between 21 February 2021 and 30 April 2021.

**Results**

Results: 141 respondents, 80.9% of whom were dentists or dental students, filled the questionnaire. 81.6% of them acquired their knowledge of sustainability from online media content. More than 80% of them consider climate change an important or high priority global problem. In both workplace and everyday sustainability, selective waste collection, energy conservation and efficiency were the most common, while reducing meat consumption, environmental activism and talking about sustainability were the least common aspects. Respondents identified selective waste collection, reducing paper use and upgrading building energetics as the most necessary improvements in workplace sustainability. Switching from single-use towards reusable dental equipment in clinical care is moderately advocated by the respondents.

**Conclusions**

Conclusions: The overall attitudes of the study sample towards sustainability are good. The responses also highlighted some gaps in this area and can help planning the necessary further steps. The combination of awareness and strong beliefs can create the motivation for conscious bottom-up actions, which is the key for an institutional shift towards sustainable dental care. Our Sustainable Dentistry Working Group has been established to catalyse these transformations.

Oral Health Interventions for Those Incarcerated, a Scoping Review
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\textsuperscript{1}Centre for Dental Public Health and Primary Care, Queen Mary University of London, London, United Kingdom, \textsuperscript{2}NIHR, London, United Kingdom

**Objectives**

To facilitate a better understanding of oral health interventions implemented in prison settings, synthesise the theoretical frameworks utilised and map the barriers and facilitators for implementation.

**Methods**

A scoping review using Joanna Briggs Institute methodology, six databases were searched; Medline (R), Emcare, Embase, AMED, Cochrane and PsychINFO. A total of 978 studies were returned and screened by abstract and title. The inclusion criteria were studies that involved interventions addressing the oral health of prisoners, published after 2000, in any language, all levels of prison security. Observational studies were excluded.

**Results**

Ten studies published between 2008 and 2021 were included. All were conducted in high-income countries. Three intervention types were demonstrated: health coaching or health education(n = 5), teledentistry(n= 3) and screening or triaging protocols(n = 2). A range of theoretical models were used to support the intervention; motivational interviewing, health education, peer coaching and health-promoting environments. The barriers and facilitators to successful implementation were grouped into a framework of four overarching themes and sub-themes. These themes included the prison environment, recruitment, incentives for compliance and staffing levels both for researchers and prison staff. Sub-themes diverged into factors such as staffing barriers, engagement with healthcare staff, competing interests, prisoner gaming, motivation, security procedures and the logistical challenges associated with unpredictable population numbers.

**Conclusions**

The prison population faces inequalities in oral health globally due to a loss of agency in accessing healthcare and risk factors predisposing them to oral disease. In order to address these challenges, successful interventions need to consider the contextually specific factors which facilitate or impede intervention implementation. A mapping of these factors is discussed in this review.
Optimisation of a Complex Intervention Reducing Inequalities in Dental Visiting

Rebecca Harris, Victoria Lowers

Public Health, Policy and Systems, The University of Liverpool, Liverpool, United Kingdom

Objectives Complex interventions may fail to show an intervention effect due to genuine ineffectiveness, sub-optimal intervention design or implementation failure - or a combination of these. Guidance therefore recommends pre-trial work is undertaken to optimise the intervention and its delivery.

Objective: To refine aspects of an opportunistic intervention delivered to adults attending for urgent dental care which aims to reduce inequalities in planned dental visiting, in order to optimise its intervention design and address potential implementation issues.

Methods Intervention material was discussed in 7 focus groups with mixed policy makers, dental nurses, dentists and community members (n=43). Embedded in a feasibility study, 58 hours of observation of intervention delivery was undertaken together with interviews with 2 dental nurses (DN) delivering the intervention, and 8 patients receiving the intervention. Stakeholder feedback/observations were captured by fieldnotes, with interviews audio-taped and analysed thematically.

Results i) Patients strongly related to the brief videos shown, finding them not just engaging, but resonating with their self-identity: “It was like looking in a mirror”. The final intervention design would therefore start with these videos. ii) Patients liked accompanying booklet information in very small chunks, but DNs found it hard to manage the separate resources as well as trial documentation, so the final intervention design had fewer separate materials. iii) The supportive conversation with the nurse was important to patients, although observations showed further training was needed to refocus DN attention away from resources and onto eliciting concerns and listening to patients. iv) Training needed to include building DN confidence and practical sessions with role-play. v) Passwords to the online material needed streamlining. vi) Final version booklets and videos needed to include more ethnically diverse characters vii) A booklet for the dentist was scaled back.

Conclusions Optimisation included emphasising impactful parts of the intervention and amending weaker ones.
Delivery of Hemichannel Blocker for Topical Treatment of Pulpitis
Wen Yi Lim1,2, Jiah Shin Chin1,3, Leigh E. Madden2,3, David Becker2,3
1National Dental Centre, Singapore, Singapore, 2Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore, 3Agency for Science, Technology and Research, Singapore, Singapore

Objectives
Connexins are trans-membrane proteins which are upregulated during pulpitis. 6 connexin proteins form a pore in the cell membrane, also known as a connexon or hemichannel, which enables the exchange of small metabolites and signalling molecules. These membranous pores increase pathologically during inflammation and can lead to increased host collateral damage. The objective of this study is to investigate use of a cis-benzopyran compound Tonabersat (SB-220453), a connexin hemichannel blocker, in the treatment of pulpitis.

Methods
For the in-vitro stage, cultured DPSCs (P3-6, Lonza Poietics) were used to assess the degree of connexin-43 and hemichannel reduction upon incubation with 10µM-300µM Tonabersat (reconstituted in DMSO). Scratch wound assay was also carried out to determine the rate of wound closure. Subsequently, we used 12-week-old male Sprague-Dawley rats to create mechanical pulp exposures, which were left untreated for 2 days. Thereafter, teeth were subjected to partial pulpotomy on the day of treatment. Tonabersat, delivered either via pluronic gel, coated-polycaprolactone or polycaprolactone/polyactic-co-glycolic acid manufactured via dip-freeze technique, were applied on rodent pulp before capping with Biodentine® (n=8 per group). Animals were sacrificed at days 1 and 5, subjected to histology and immunofluorescent staining to examine degree of hemichannel and connexin downregulation, as well as inflammation markers- MPO and NLRP3. Kruskal-Wallis analysis was performed.

Results
Tonabersat effectively reduced hemichannel expression at 300µM resulting in a notable increased rate of wound closure in-vitro. It was observed that the DMSO vehicle control in all 3 scaffolds had a negative effect, increasing inflammatory markers as well as demonstrating poorer H&E scores. Tonabersat delivered in all three scaffolds however, showed promising histological outcomes, and corresponding favourable reduction of hemichannel expression, further confirmed by reduced levels of MPO and NLRP3.

Conclusions
Future work employing Tonabersat for treatment of pulpitis would entail the exploration of other vehicle carriers and evaluating oral delivery methods.

Experimental Borosilicate Bioactive Glasses: Pulp Cells Behaviour and Mechanical Characterisation
Nina Attik1, Federico Lizzi1, Christelle Goutaudier1, Phil Jackson1, Ian Campbell2, Sébastien Rizzo1, Brigitte Grosgogeat1, Cyril Villat1
1Université Claude Bernard, Lyon1, Faculté d’odontologie de Lyon, Lyon, France, 2Lucideon, Stoke-on-Trent, United Kingdom

Objectives
To assess the effect of two experimental phase-separated borosilicate (PSBS) one without alumina (PSBS8) and one containing alumina (PSBS16) on dental pulp cells; and to compare their mechanical properties to a conventional fluoroaluminosilicate glass (GIC).

Methods
The cytocompatibility assessment of the two borosilicate glasses was achieved on cultured primary human pulp cells (DPSCs). Alamar blue assay was used to assess cell metabolic activity, and cell morphology was evaluated by confocal imaging. The bioactivity in stimulated body fluid was evaluated using SEM-EDX analysis. Vickers microhardness and flexural strength were assessed after incorporating the glass particles into the conventional GIC liquid. One-way Analysis of Variance (ANOVA) followed by Post Hoc tests were used for statistical analysis.

Results
The two borosilicate glasses enhanced HDPs cells behavior. PSBS8 was more efficient than the PSBS16 and the GIC glasses regardless of contact type and time point. EDX analysis confirmed an initial Ca/P ratio (2.1 for 4SS5K and 2.08 for PSBS8) after 3 weeks of immersion. After 42 days of maturation, the cement prepared using PSBS8 showed significantly higher Vickers hardness values (p=0.001) than that prepared using PSBS16 (HV=47±7 vs 37 ±9). Regarding the flexural strength, after 24 hours of maturation PSBS8 exhibited a flexural strength of 14.2 MPa compared to 5.4 MPa for PSBS16 and 16.4 MPa for GIC. However, both borosilicate glasses demonstrated lower mechanical properties compared to the GIC glass.

Conclusions
The present in vitro results demonstrated that the experimental borosilicate bioactive glass without alumina enhanced pulp cells behaviour better than the tested conventional glass ionomer cement and the experimental borosilicate glass containing alumina. In the context of tissue preservation and minimally invasive dentistry, the experimental borosilicate glasses deserves to be further developed for a potential use as filler in the development of new bioactive materials for endodontic and/or restorative dentistry.

PER-IADR Oral Health Congress 2022 in Marseille 29
An Innovative Hydrogel Incorporating Antibacterial Nanoparticles to Regenerate Dental Pulp

Mourad Bekhouche1,2, Fanny Charriaud3, Elie Daou2, Marie Bolon2, Myriam Lamrayah2, Arthur Costantini1,2, Stephanie Gobert3, Marielle Pasdeloup2, Arthur Verrier3, Brigitte Alliot-Licht3, Maxime Ducret1,2, Jean-Christophe Farges1,2
1Faculty of Odontology, University Lyon 1, Lyon, France, 2Laboratory of Tissue Biology and therapeutics Engineering, UMR5305, Lyon, France, 3Faculty of Odontology, University of Nantes, Nantes, France

Objectives Regenerating a functional dental pulp (DP) in the pulpectomized root canal was recently proposed as a novel therapeutic strategy in human endodontics. However, residual endodontic bacteria can hinder DP regeneration. In this context, designing antimicrobial scaffolds able to eliminate bacteria, while being not deleterious for DP-mesenchymal stem cell (DP-MSC) survival and pulp tissue regeneration, is needed. Our aim was to develop an innovative hydrogel incorporating clindamycin (CLIN)-releasing poly (D,L) lactic acid (PLA)-nanoparticles (CLIN/PLA-NPs) to confer this scaffold antibacterial property.

Methods PLA-NPs and CLIN/PLA-NPs were synthesized by the solvent diffusion method. NP diameter and polydispersity index (PDI) were determined by dynamic light scattering, NP zeta potential by electrophoretic mobility and NP morphology by scanning electron microscopy. Efficiency of CLIN entrapment into PLA-NPs was determined by high performance liquid chromatography. NP release from the hydrogel was assessed using fluorescent PLA-NPs. Antibacterial activity of fibrin hydrogels containing CLIN/PLA-NPs, PLA-NPs or CLIN alone, was determined on Enterococcus faecalis using the agar diffusion method. Human DP-MSCs and CLIN/PLA-NPs or CLIN alone were co-incorporated into 10mg/mL fibrin hydrogels to get a final CLIN concentration of 50µg/mL hydrogel. DP-MSC viability was determined with a Live/Deadkit in fibrin-alone, CLIN/PLA-NP/fibrin, PLA-NP/fibrin and CLIN/fibrin hydrogels.

Results PLA-NPs and CLIN/PLA-NPs showed similar diameter, PDI and zeta potential, and a spherical morphology. CLIN entrapment efficiency was about 42%. The release of fluorescent NPs from the fibrin hydrogel was of 3% after 24h. Fibrin hydrogels incorporating CLIN/PLA-NPs or CLIN alone had similar antibacterial activity. DP-MSC viability into the hydrogels was similar (about 75%) in the 4 conditions tested.

Conclusions Incorporating CLIN/PLA-NPs within a fibrin hydrogel could be beneficial to promote DP regeneration thanks to the antibacterial effect of the CLIN/PLA-NPs and the absence of significant detrimental effect of CLIN, PLA-NPs and CLIN/PLA-NPs on DP-MSC viability.
**Autocrine Pulp Fibroblast Function Modulation via Complement C3a Local Production**

Charlotte Jeanneau¹, Thomas Giraud¹,², Imad About¹

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**Objectives** Local Complement activation in dental tissues plays a major role, not only in eliminating bacteria but also in the initial steps of dental tissue regeneration. C3a is the first active molecule secreted by pulp fibroblasts after Complement activation. Recent data demonstrated that this active molecule mobilizes pulp stem cells (DPSCs) and provides a gradient for pulp fibroblast recruitment. This work was designed to investigate C3a autocrine modulatory effect on pulp fibroblasts growth factors secretion and, subsequently, on fibroblast proliferation and DPSCs recruitment.

**Methods** Human pulp cells were isolated from third molars. DPSCs were separated from fibroblasts using magnetic cell sorting with STRO-1. Pulp fibroblasts were either stimulated with C3a (200ng/ml) or incubated with 1µg/ml Lipoteichoic Acid (LTA) to simulate a carious lesion. After 48h, the secretion of FGF2, TGFb1 and VEGF by pulp fibroblasts (± C3a specific antagonist) was quantified by ELISA. After this stimulation, the resulting supernatants were harvested and used to investigate their effects on unstimulated pulp fibroblasts proliferation with the MTT test. DPSCs recruitment towards the stimulated fibroblasts was investigated in Boyden chambers.

**Results** Both cell incubation with LTA or stimulation with C3a significantly increased FGF2, TGFb1 and VEGF secretion. The supernatants obtained after both stimulation methods significantly induced fibroblast proliferation as compared to unstimulated cell supernatants or after cell incubation of with the C3a antagonist. An important level of DPSCs recruitment was observed with LTA-stimulated and C3a-stimulated fibroblast supernatants as compared with the unstimulated and with supernatants of fibroblasts incubated with the C3a antagonist.

**Conclusions** Within the limits of this investigation, C3a complement fragment stimulate growth factor secretion by pulp fibroblasts and modulate fibroblast proliferation and DPSC recruitment. This autocrine stimulation of pulp fibroblasts is a new step in understanding the link between pulp tissue injury/inflammation and regeneration.
Dentin-Pulp Complex Regeneration: Towards a Paradigm Shift for Dental Practice

Foteini Machla¹, Viktoriya Sokolova², Varvara Platania³, Oleg Prymak², Kathrin Kostka², Benedikt Kruse², Sofia Pasadaki⁴, Kalliopi Alpantaki⁵, Marina Vidaki⁵, Maria Chatzinikolaidou⁵,⁶, Matthias Epple², Athina Bakopoulou¹

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Objectives The status quo for the replacement of the bulk of defected dentin combined with vital pulp therapeutics (VPT) is based on inert biomaterials. This study provides a novel tissue-engineering-based approach for the structural and functional regeneration of the dentin-pulp complex. Human dentin scaffolds (hFDDSs) were impregnated with calcium phosphate nanoparticles (CaP-NPs) encoding for morphogenetic proteins, with perspectives to be applied in a digital dentistry workflow.

Methods The hFDDSs were EDTA-treated, freeze-dried, and characterized by X-ray powder diffraction (XRD), confocal laser scanning microscopy (CLSM), and scanning electron microscopy (SEM). The CaP-NPs conjugated with plasmids encoding for Dentin Matrix Protein-1 (pDMP1) or Bone Morphogenetic Protein-2 (pBMP2) were synthesized, characterized, and tested in terms of biocompatibility (MTT assay), cellular uptake, and transfection efficiency [flow cytometry, transmission electron microscopy (TEM) and CLSM] on dental pulp stem cells (DPSCs). The construct of hFDDSs impregnated with NPs (NPs-hFDDSs) was tested for cytotoxicity (live/dead staining), and foreign body reaction in vivo (C57BL mice, N=4). Polarization of cells was evaluated by SEM, while odontogenic differentiation by qPCR.

Results Based on the biocompatibility vs. transfectability results, nanoparticle concentration of 4-μg-Ca/mL was selected to prepare the NPs-hFDDSs. Biocompatibility of NPs-hFDDSs was similar to the control. In vivo experiments showed no foreign body reaction against the construct. DPSCs differentiated into polarized odontoblast-like cells on NPs-hFDDSs, upregulating the expression of DMP1, DSPP and BMP2, while downregulating ALP and RunX2. The differentiation induction by pDMP1- and pBMP2-NPs-hFDDSs was significantly higher than the positive control (dexamethasone). SEM revealed polarization of the differentiated cells, with processes extending into the natural dentinal tubules.

Conclusions The proposed approach provides a promising bioactive dentin substitute and VPT tool that should be considered for clinical application in cases of deep lesions coming in direct/indirect contact with the pulp, promoting tissue healing and reparative dentin formation (Figure 1).

Figure 1. Clinical perspectives of the study. Dentin defected tissue is removed by mechanical means (A), the prepared cavity is scanned with an intra-oral scanner (B), the hFDDS is cut to the proper dimensions using CAD-CAM technologies (C), and the NPs-hFDDS inlay is placed in the cavity (D). The inlay can be fixed against the vertical dentin walls of the cavity using a bioactive cement, while the lower side of the hFDDS carrying the pDMP1-NPs and pBMP2-NPs is in direct contact with the exposed pulp. The coronal part of the cavity is sealed with a temporary filling material, until permanent restoration.
**Synergistic Effects of SDF and Hypochlorite on Primary Dentine Lesions**

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**Objectives** The aim was to investigate the effects of sodium hypochlorite pre-treatment on primary molar dentine artificial carious lesions undergoing silver diamine fluoride (SDF) treatment using X-ray microtomography (XMT) and Backscattered scanning electron microscopy (BSE).

**Methods** Five extracted primary molar teeth were sectioned to create five control and five test blocks. The occlusal enamel was removed and the blocks varnished to reveal a 2x2mm window of occlusal dentine. The blocks were demineralised for 24 in acetic acid (0.1M, pH 4) to create standardised artificial carious lesions. The test blocks underwent 120s sodium hypochlorite (5%) pre-treatment followed by SDF treatment (Riva Star). The control blocks received SDF treatment. All blocks were then remineralised (CaCl2, KH2PO4, NaCl) for 5 days. XMT (60kV, 20 hours) was used to obtain 3D images of the blocks at baseline, before and after remineralisation to compare mineral deposition and backscattered electron microscopy (BSE) was used to confirm the silver distribution.

**Results** XMT showed that the test group showed higher mineral density values (LAC 3.22) at the artificial lesion surface than the control group (LAC 1.51). Higher silver concentrations were seen in the body of the remineralised lesion in the control group (LAC 1.25) than the test group (LAC 1.0). A ‘sub lesion zone’ of silver deposition was more distinct in the test group 100-200μm below the lesion base.

BSE imaging revealed surface silver was seen in the test group that projected from within the dentine tubules beyond the occlusal border of dentine (10-20μm). The dentine tubules within the sub lesion zone below the lesion base were more saturated with silver particles in the test group.

**Conclusions** Sodium hypochlorite pre-treatment of artificial dentine carious lesions may operate synergistically with SDF to enhance its protective and caries arrest properties.
Piloting of an Individualized Dental Training Program for Autistic Children
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Objectives To develop and test an individualized dental training program for children with autism spectrum disorder

Methods An individualized dental training program for children with ASD consisting of a 10-step desensitization protocol (from entering the dental office to taking X-ray pictures) accompanied with a tool kit (pictures, social stories, movies, dental examination equipment) for in-school preparations was developed based on existing literature. The program was developed in collaboration with educators at a special-needs class for children with ASD. The program consisted of up to 10 weekly sessions at a regular dentists’ office. The rate of progression was adapted to fit the individual child. Individual preparations prior to the sessions were included in the weekly school schedule and were done by the children’s educators. On the 10th session, all accomplished steps were performed by a different dentist. To assess acceptance, cooperation was measured using Frankl scale.

Results Seventeen children (age 7-13) were included in the pilot study. The group of children was highly heterogenous with regards to age, speech development, language comprehension, sensitivities, and previous experiences with dental examinations. Fourteen of the children accepted a full examination after attending the program. The use and preferences of preparatory tools and the progression rate through the 10-step protocol varied between the children. The number of children accepting examination with mirror and probe and taking an X-ray after completion of the program increased compared to parents reports on previous achievements. Changing the dentist did not lead to markedly reduced cooperation.

Conclusions An individualized dental training program consisting of thorough preparations and gradual in-clinic exposure may increase acceptance towards dental procedures in a heterogenous group of children with ASD and facilitate the conduct of a dental examination.

The Association Between Multisystem Inflammatory Syndrome and Oral Health Status in Children
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Objectives The aim of this cross-sectional study was to investigate oral health status in children with Multisystem Inflammatory Syndrome (MIS-C) compared to children with SARS-CoV-2 infection.

Methods A total of 54 children with COVID-19 associated MIS-C (n=23) and mild COVID-19 (n=31) were recruited to the study (Age range: 5-15; gender (F/M): 23/31). Sociodemographic variables, medical examinations, oral hygiene habits as well as extra- and intra-oral findings (DMFT/dmft index, OHI scores and oral mucosal changes) were recorded.

Results MIS-C was associated with cracked lips (all patients with MIS-C) and oral mucosal changes including erythema, white lesion and strawberry tongue and swelling of the gingiva compared to COVID-19 group (frequency of more than one mucosal change: 100% vs 35%) (p<0.001). MIS-C led to 45.8% increase in DMFT/dmft scores higher than 6 (DMFT/dmft (mean±SD): 5.52±3.16 (MIS-C) versus 2.26±1.80 (COVID-19) (p<0.01). Elevated OHI scores was associated with MIS-C (mean±SD: 3.06±1.02 (MIS-C) versus 2.41±0.97 (COVID-19) as well (p<0.05).

Conclusions Oral manifestations mainly strawberry and erythematosus tongue were predominantly associated with MIS-C. Oral/dental symptoms among children with MIS were prevalent. Therefore, dental professionals should be aware of the oral manifestations to associate to MIS-C which may have high mortality and morbidity rate.
Sugar, Environment, and Local Paediatric Dental Needs
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Objectives To examine the relationship between sugar promoting areas in a local authority of South-East England and the dental needs of 5-to-11-year-old patients attending a primary dental care setting in the area.

Methods Secondary analyses of cross-sectional, open-access data on dental records (2008-2012) of paediatric patients visiting the University of Portsmouth Dental Academy. These data informing on age, gender, and tooth extraction, were linked to the newly developed Index of Sugar Promoting Environment Affecting Child Dental Health (ISPE-ACDH), via census-based Middle-layer Super Output Areas (MSOAs) of patients’ residences. The ISPE-ACDH measures the distribution of socio-environmental factors of sugar consumption amongst children living in England and provides standardized scores for the overall index and component domains including, neighbourhood-, school-, and family-environment based on sugar promotion levels in MSOAs. Analyses involved descriptive, univariate, and multivariate logistic regression.

Results Data comprised 429-patients (mean-age:7.78, SD:1.976), Male vs Female:(50.1% vs 49.9%). Seventy patients had undergone tooth extraction. Scores for MSOAs (n=23): index (mean:1.184, SD:0.645), neighbourhood-domain (mean:1.187, SD:0.647), school-domain (mean:1.073, SD:0.600), family-domain (mean:0.838, SD:0.724). Univariate models demonstrated age as a significant predictor of tooth extraction (p<0.05). A multivariate logistic regression, controlling for age and gender, demonstrated that the index was insignificantly associated with tooth extraction. Another model, adjusted for age, gender, and domain scores, revealed that the likelihood of tooth extraction, significantly increased by 16% with age, and 350% in patients from MSOAs with higher sugar promotion in schools, and declined by 38% in patients from MSOAs with higher proportions of families experiencing poor socioeconomic conditions.

Conclusions This study quantifies how child dental needs can be influenced by sugar promoting environments. This is defined by the factors in school and home environments and explained by a newly developed index. These associations need further exploration at the national level to support targeted sugar reduction programmes.
Effect of air-Particle Abrasion on Reliability of Additively-Manufactured Zirconia Ceramics

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Objectives Due to its excellent accuracy and capability to produce dense objects, stereolithography (SLA) is the most promising additive manufacturing technology for zirconia ceramics. Still, surface finish can be compromised, which may affect materials’ strength. Also, the strength may be altered when adhesion-promoting surface treatments, i.e., air-particle abrasion (APA), are employed, which are even more critical for newer translucent zirconia. The aim was to evaluate and compare the effects of APA on mechanical properties of different zirconia ceramics fabricated with SLA and dry-pressing (DP).

Methods Disk-shaped specimens (n=90/gp) were fabricated from 3%mol. (3Y) and 5%mol. (5Y) yttria-zirconia by SLA (CeraFab 7500; Lithoz GmbH, Vienna, Austria; lateral resolution of 40 μm and layer thickness of 25 μm) and uniaxially DP (147 MPa, 30 s) that served as control. Top surface of specimens (n=60) was treated by APA with 50 μm Al2O3 at 2 bars. Half of APA specimens were treated with regeneration firing (RF; 1000°C, 15 min). Control specimens (n=30) were left in as-sintered state. Biaxial flexural strength was determined (ball-on-three-balls method; 1 mm/min) coupled with Weibull analysis and fractography (SEM). XRD and FIB-SEM determined crystal phases and depth of transformation zone (TZD), respectively. ANOVA with Tukey’s post hoc and t-test was used (p<0.05).

Results SLA zirconia exhibited lower characteristic strength (σθ) and reliability (m), regardless of zirconia type (3Y, 5Y). The APA increased σθ and m of 3Y and decreased σθ of 5Y material, whereas m was increased, regardless of fabrication method. No differences between SLA and DP zirconia were noted regarding crystal phase structure and TZD. The prevailing critical flaws for SLA and DP were process-related; size of flaws was substantially larger in SLA.

Conclusions Compared to DP, SLA zirconia presented mechanically inferiorly, still reaching clinically-acceptable strength; whereas APA effects did not differ between SLA and DP zirconia. Main observed defects were process-related.

CAD/CAM-Milled and 3D-Printed Monolithic Zirconia Crown Retention

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Objectives To determine retention of monolithic zirconia crowns adhesively luted onto different tooth-preparation configurations with varying levels of macro-retention and to test retention of an experimentally developed micro-retentive design at the inner restoration side of 3D-printed monolithic zirconia crowns.

Methods Sixty recently extracted human maxillary molars were prepared following three tooth-prep designs with different macro-retention level: (1) no macro-retention (flat surface); (2) prep with a 2-mm abutment height; (3) prep with a 4-mm abutment height. The preps were digitally scanned to design the crowns, enabling also to measure the whole prep area and the axial wall friction area. Thirty-six crowns (n=12) were milled using a chairside Cerec MC XL milling unit (Dentsply Sirona) and 24 crowns were 3D-printed using an inkjet XJet Carmel 1400C (XJet) 3D-printer. Twelve of the printed crowns received an inner surface micro-retention design. Crowns were adhesively luted using the self-etch adhesive-assisted composite cement Panavia V5 (Kuraray Noritake) and were pulled off from the tooth abutment using a material tester (5848 MicroTester, Instron). The pull-off force was recorded with crown retention determined by dividing it by the total surface area (N/mm²). Statistical significance was assessed with the independent-samples Kruskal-Wallis test (α=.05) with Bonferroni correction for multiple tests.

Results The pull-off force significantly increased with increasing prep-surface area, while no difference was found by increasing friction area. No significant difference in crown retention was found between the milled and XJet-printed full crowns, while the 3D-printed micro-retentive inner surface design significantly increased crown retention when compared to the milling group.

Conclusions Although a larger surface area increased crown retention, a larger friction area did not increase crown retention. Incorporating a micro-retentive inner surface design within the walls of 3D-printed crowns effectively increased crown retention.
Objectives To evaluate two-body wear of two pre-colored highly translucent multilayer monolithic zirconia ceramics, which were colored using two different techniques.

Methods Two pre-sintered 5 mol% yttria partially stabilized zirconia (5Y-PSZ) disks (Lava Esthetic A2, 3M Oral Care; Katana STML A2, Kuraray Noritake) were cut in plates, sintered according to the manufacturer’s instructions and mirror-polished. The pigment composition and distribution were thoroughly evaluated by light and fluorescence microscopy, electron probe micro-analyzer (EPMA) with WDS and nano-SEM with energy-dispersive X-ray spectroscopy (EDX). Chemical and phase composition were assessed using X-ray fluorescence (XRF) and X-ray diffraction (XRD) along with electron backscatter diffraction (EBSD), respectively. Samples were then loaded in the chewing simulator (5 kg vertical load, 2.1 Hz) in water of 5-55 °C for 800 000 cycles against steatite antagonists. Wear tracks on the zirconia ceramics were scanned with a non-contact 3D laser profilometer and super-impositions were used to determine wear loss of the antagonists. Wear surfaces were analyzed by nano-SEM.

Results Pigments in Lava Esthetic were seen as large yellow and blue, fluorescent agglomerates consisting of small grains (0.54 µm and 0.36 µm, versus 0.86 µm for the surrounding grains) with a lower Y2O3 content and the presence of Er, Hf and Al. The coloring agglomerates however were not observed in Katana STML. Nevertheless, Lava Esthetic and Katana STML showed similar wear trend and similar amount of wear. Laser profilometry confirmed that both ceramics worn similarly (= 19 µm in depth). While abrasive wear without obvious cracks was seen in both zirconia ceramics, limited amounts of monoclinic zirconia were detected in the worn surfaces. Lava Esthetics was characterized with wear degradation occurring exclusively within the pigment agglomerates. Furthermore, no significant differences in antagonist wear were found between both Lava Esthetic and Katana STML (4.62±0.11 and 4.68±0.09 mm³, respectively).

Conclusions In the context of this study, Lava Esthetic and Katana STML, although being colored using different techniques, showed similar wear behaviors.

A Novel Method for Pretreating Zirconia Resin-Bonded Fixed Dental Prostheses

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Objectives Debonding of zirconia cantilevered resin-bonded fixed dental prostheses (RBFDPs) remains the main treatment complication. Nanostructured alumina coating (NAC) provides a promising pretreatment of zirconia bonding surface, which does not damage the zirconia surface. The aim of the study was to “in vitro” and “in vivo” evaluate the effect of NAC on zirconia RBFDPs and compare it to clinically established airborne-particle abrasion (APA).

Methods For studying the effect of NAC “in vitro”, the bonding performances of RBFDPs bonded on complementary zirconia incisors with both substrates pretreated with NAC or APA were compared after 150 days of water storage and thermomechanical aging (37.500 cycles, 5-55 °C; 50N/1.2×10⁶ cycles) by measuring load-bearing capacity (LBC) (T-test, ANOVA, Tukey’s, P<0.05). For studying NAC “in vivo”, the success and survival rates of NAC and APA pretreated RBFDPs were compared in a prospective randomized, controlled, double-blind clinical trial (Kaplan-Meier, log-rank test, P<0.05).

Results Before aging, NAC RBFDPs provided superior mean LBC (724 ±58 N) to APA (564 ±31 N), while after aging, no differences were detected with the LBC approximately 590 N (P>0.05). After a mean ±standard deviation observation period of 33.1 ±12.9 months (maximum 52.8 months), 33 participants with 37 restorations were examined. The survival rates for both groups were 100%, while the success rate was 87.4% for the NAC and 80.2% for the APA group, with no significant differences (P>0.05).

Conclusions NAC could be regarded as a viable alternative pretreatment method for zirconia RBFDPs, especially when fabricated from translucent zirconia, which is more susceptible to the damaging effect of APA.
Fracture Strength Evaluation of CAD/CAM Endocrowns After Thermo-Mechanical Aging

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Objectives This in vitro study aimed to evaluate the fracture strength (FS; N) of glass-ceramic, hybrid ceramic, and composite CAD/CAM endocrowns in molars after thermo-mechanical aging.

Methods 70 human non-carious third molars with similar crown size and shape were randomly divided into seven groups according to the CAD/CAM material used for the fabrication of endocrown restorations. Intact teeth without cavity preparation were used as control (n=10), while 60 teeth were endodontically treated. After endodontic treatment, standardized endocrown preparations were prepared with a 2 mm deep central retention cavity in the pulp chamber, 2mm thick axial walls, and 90° circular butt joint margins. Endocrowns (n=10 in each group) were produced from feldspathic (Cerec Blocks, Sirona, C), glazed (CDG), and milled (CDM) zirconia reinforced lithium silicate (Celtra Duo, Dentsply), leucite-reinforced feldspar ceramic (LRF Initial, GC, LRF), hybrid ceramic (Cerasmart270, GC, CS) and composite (Grandio Blocs, Voco, G) CAD/CAM materials and adhesively luted with a universal adhesive (Futurabond U; VoCo) and dual-cure resin cement (Bifix QM);(Demi Ultra; Kerr). Following thermocycling for 20,000 cycles and 480000 load cycles in a chewing simulator (CS-4.2, SD Mechatronik), FS was evaluated (Instron). Data were analyzed with one-way ANOVA and post hoc Tukey’s tests (p<0.05).

Results FS was significantly influenced by the CAD/CAM material (p=0.00). CS and CDG had significantly the highest FS, which were not significantly different from the control group (p>0.05), while there were no significant differences between LRF, G, and CDM (p>0.05) that were significantly higher than C (p<0.05). The majority of the failures in CS, CDG, and G groups were repairable, while in C were non-repairable.

Conclusions Hybrid ceramic and glazed zirconia reinforced lithium silicate glass-ceramic CAD/CAM endocrowns exhibited similar fracture strength as the intact molars. In contrast, feldspathic endocrowns resulted in more irreparable fractures with lower fracture strength.
Ion-Release and Antibacterial Properties of Copper-Doped Bioactive Glass Containing Composites

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Objectives This study evaluated the ion-release and antibacterial effect against Streptococcus Mutans of newly developed experimental resin composites containing copper-doped mesoporous bioactive glass nanospheres (Cu-MBGN).

Methods Cu-MBGN was synthesized using microemulsion-assisted sol-gel method. Eight experimental composites were designed using Bis-GMA/TEGDMA base. Cu-MBGN composites had 1, 5, or 10wt.% of Cu-MBGN, silanized nanosilica, and inert silanized microfillers up to 65wt% or 70wt%. Composites with 45S5 bioactive glass or silica were mixed as reference materials. Discoid specimens (d=3mm, h=2mm, n=3/gr) were light-cured (2x20s, 890 mW/cm²). The specimens were immersed in HEPES buffered solution for 28 days, and the calcium and copper ion release was measured with ICP-MS. The adherence of S.mutans to test materials was determined in a free-floating and biofilm-forming test setup. SEM-EDX analysis was performed to determine the filler particle and elemental distribution at the surface. Nano-CT was used to characterize the percentage of composites’ total, open and closed porosities. The data were analyzed using two-way ANOVA and Tukey’s post-hoc test (α=0.05).

Results Expectedly, only Cu-MBGN materials showed the dose-dependent Cu-release, with the highest values at 10% Cu-MBGN and the lowest at 1% Cu-MBGN. The highest Cu-release was achieved at 1-day, followed by a significant drop on days 3, 7, and 14, and another rise at 28-days. Accordingly, 10% Cu-MBGN had the best antibacterial effect, demonstrated by the lowest adherence of free-floating bacteria and the lowest biofilm formation. 45S5-containing materials showed, in contrast, the highest S.mutans adherence. Ca-release was the highest in bioactive controls containing 15% of 45S5 BG, which correlated with the highest number of open porosities at the surface. Cu-MBGN composites demonstrated the same dose-dependent behavior for Ca as for Cu, with a dominant spike at 1-day. SEM-EDX showed a homogeneous filler distribution of all the materials.

Conclusions Cu-MBGN composites show better antibacterial properties than 45S5 BG composites but a sustained Ca-release.
Anti-Bacterial Composite Inhibits Growth of Salivary Bacteria: an Ex-Vivo Study
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Objectives: Studies suggest that the incidence of secondary caries is higher in composite restorations than in amalgams. Saliva contains a broad spectrum of endogenous bacteria, some of which are cariogenic bacteria that interact to form the dental plaque. The aim of this study is to test the ability of an anti-bacterial composite to inhibit the growth of this wide variety of salivary bacteria thus affect the occurrence of secondary caries.

Methods: Test was carried out in a 96-well, flat-bottom microtiter plate. Bottom of 4 wells were coated with tested restorative materials: Anti-bacterial composite Infinix (Nobio), composite Filtek (3M) and amalgam Spherodon-M (Silmet). Wells were rinsed with phosphate-buffered saline. Unstimulated saliva was collected from 30 participants and tested separately. Whole saliva (10µl) from each participant was placed at the center of each tested materials. Plate was incubated (1h, 37°C) to ensure interaction between salivary bacteria and tested material. Growth medium was added, then plate was incubated again (1h, 37°C). Supernatant was transferred to a new plate. Plate was inserted into a microplate spectrophotometer (20h, 37°C). Kinetic reading was taken every 20min, optical density was measured as an indicator of bacterial growth. Measurements were analyzed and plotted.

Results: Bacterial growth rate measured in wells of bacteria that interacted with anti-bacterial composite was on average 4.87 times slower compared to control composite and 3.86 slower compared to amalgam (p<0.001). Moreover, growth onset of bacteria at the same wells, was delayed compared to control composite and amalgam (2.25 times and 1.59 times respectively; p<0.01).

Conclusions: Anti-bacterial composite showed statistically significant inhibition of salivary bacteria growth. Various levels of this growth inhibition were observed in samples collected from 30 participants thus indicating the anti-bacterial composite has the potential to shift caries balance towards health in all participants.

Effect of Particle Size on Bioactivity of Novel Dental Composite.
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Objectives: Bioactive glass (BAG) is well-known to possess antimicrobial and remineralising properties. By incorporating BAG in dental restorative materials, the release of remineralising ions will promote the formation of apatite. This is a desirable property at the tooth-restoration interface, as it protects against secondary caries and minimizes any microleakage due to polymerization shrinkage. The aim of this study is to investigate the effect of different particle sizes of novel BAG to form apatite.

Methods: Three Bioactive Glasses with different particle sizes (D50= 4µm, 8µm,12µm) containing SiO2-P2O5-CaO-CaF2-Na2O-K2O-MgO-SrO-ZnO were prepared using the melt quench technique at 1400°C. Glass powder added in the resin matrix composed of BisEMA, UDMA, and TEGDMA and light-cured for the 20s. Samples were stored at 37°C in different Artificial Saliva pH=7, pH=4, and TRIS-Buffer (AS7, AS4, and TRIS) for varying time intervals (1,3,7,30,90,180days). All synthesized samples were analyzed by doing pH measurement, Fourier Transform Infrared Spectroscopy (FTIR), Degree of conversion, and X-ray Diffraction (XRD).

Results: FTIR spectra and XRD patterns of all bioactive composites showed characteristic peaks for the formation of apatite in AS4, AS7, and TRIS which increases with time. 4µm is the most reactive and forms apatite relatively quicker compared to 8µm and 12µm. The degree of conversion of dental composite ranges between 66-76% in the order of 4 µm >8 µm >12µm. All samples showed an increase in pH favorable for apatite precipitation, especially in AS4 within 24hrs which corresponds to the XRD and FTIR results.

Conclusions: It appeared that this novel small size of BAG filler particles can have rapid apatite formation in Artificial saliva and Tris-buffer. Hence, it can be used as a smart clinical restorative material that can neutralize acid challenges and promote remineralization with enhanced biomineralization properties.
Essential Role of HEMA in Remineralisation of Resin-Based Cements
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Objectives This study aimed at monitoring the degree of apatite formation in a range of experimental RMGIC formulations following immersion in artificial saliva.

Methods Novel RMGICs with relatively low phosphate content and different HEMA concentrations (30% and 15%), with and without substitution of the experimental glass (10 wt%) with experimental sodium-free BAG, and different polyacrylic acid (PAA) concentrations (20%, 10% and 0%), were examined together with an experimental GIC and a commercial restorative material (ACTIVA™ BioACTIVE-RESTORATIVE™). Set cement disks (n=3) were immersed in artificial saliva (AS) for 2-weeks, 1-month, 3-months, 6-months, and 12-months. 31P Magic-Angle-Spinning-Nuclear-Magnetic-Resonance (31P MAS-NMR) and 19F MAS-NMR were used to determine the ability of these cements to form apatite. Controlled water absorption experiment was conducted up to 6 months in AS, to evaluate the impact of the materials’ hydrophilicity on apatite formation.

Results 31P MAS-NMR and 19F MAS-NMR of the experimental compositions demonstrated evidence of increasing apatite formation over time. No significant difference in apatite formation was found between the experimental RMGICs and the different HEMA and PAA concentrations, while the hydrophilicity of experimental compositions had a limited effect. The substitution of 10 wt% of the experimental glass with experimental BAG accelerated apatite formation. On the contrary, the commercial material showed no signs of apatite formation, at 12-months immersion in AS.

Conclusions Experimental RMGICs and GICs formed apatite, following immersion in AS, with time. The potential of RMGICs to remineralise was higher than with GICs under the same circumstances. Presence of HEMA played a role in enhancing apatite formation within RMGICs. This could be beneficial to occlude microleakage at the tooth restoration interface and prevent secondary caries development.

Mechanical Properties of Experimental Composites With a Customized Bioactive Glass
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Objectives The aim of this in vitro study was to investigate the effect of a customized low-phosphate-containing bioactive glass (F-BG) on the mechanical properties of experimental resin composites by comparing it to the conventional bioactive glass 45S5 (C-BG).

Methods Two series of experimental light-curable resin composites were prepared by admixing 5, 10, 20, and 40 wt% of either C-BG or F-BG into a Bis-GMA/TEGDMA (70:30 wt%/wt%) resin matrix. Reinforcing fillers were added up to a total of 70 wt%. The composites were light-cured to obtain 2x2x16 mm sticks for measuring flexural strength and modulus using the three-point bending test. To evaluate the effects of aging on the degradation of mechanical properties, separate groups of specimens (n=20 per experimental group and time point) were prepared for testing after the following artificial aging protocols: 1 day (water, 37 °C), 30 days (water, 37 °C), and 30 days (water, 37 °C) + 10,000 thermocycles between 5 – 55 °C. The data were analyzed using a mixed model ANOVA and Weibull statistics.

Results A dose-dependent reduction in flexural strength and modulus was observed for both experimental composite series; however, the reduction was significantly less extensive for the composites functionalized with F-BG. The ISO 4049 requirement for a minimum flexural strength (80 MPa after 1 day in water) was fulfilled for up to 20 wt% of C-BG and 40 wt% of F-BG. Aging-induced degradation of mechanical properties was less extensive for the F-BG composites compared to the corresponding C-BG composites. Weibull analysis showed highly material-dependent reliability, which was generally reduced as the amount of bioactive glass was increased.

Conclusions Incorporating the customized low-phosphate-containing bioactive glass as a functional filler in ion-releasing resin composites resulted in better mechanical properties compared to the corresponding materials functionalized with the conventional bioactive glass 45S5.
Effect of Ion-Releasing Materials on Dentine Surface Microhardness and Appearance

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Objectives The aim of this study was to determine the effect of ion-releasing materials on dentine microhardness and surface appearance.

Methods Five materials were tested; glass ionomer cement (GC Fuji TRIAGE®), two glass hybrid cements (EQUIA Forte®HT, Riva SC), calcium silicate-based cement (Biodentine®) and an alkasite (Cention®Forte). For the control group, conventional resin composite (3M™ Filtek™ Universal) was used. A class-I cavity (3×1.5mm wide, 0.5mm deep) was made on the exposed occlusal surfaces of 60 extracted human third molars which were then demineralized by immersing them in a prepared solution (pH 5.0, 37°C) for 2 weeks, followed by placing them in saline for 2 and 4 weeks respectively. The teeth were then cut with a diamond saw perpendicular to the joint of material and demineralized surface, obtaining 10 samples for each group (n=10). Microhardness was determined by the Vickers method in triplicate. The results were analysed using SPSS statistical software and compared using analysis of variance and appropriate post-hoc tests for comparison. The statistical significance level was set to 0.05.

Results Mean microhardness values (HV0,1) obtained after 14 days were significantly different between most groups (p<0.001), with several exceptions (Biodentine® vs. Cention® Forte p = 0.08, Biodentine® vs. Riva SC p = 0.997, Riva SC vs. Cention® Forte p = 0.229). Similarly, after 28 days there were statistically significant differences between all groups (p<0.001), except EQUIA Forte®HT and GC Fuji TRIAGE® (p = 0.514) and Cention®Forte and Riva Self Cure (p = 0.687). All tested materials lead to increase in microhardness; the highest values were obtained for EQUIA Forte®HT. SEM analysis showed uneven patterns, material deposits and debris on the examined surfaces.

Conclusions All tested materials lead to certain mineral gain and significantly affected the appearance of dentine surface. EQUIA Forte®HT and GC Fuji TRIAGE® caused the biggest increase in microhardness.

The Remineralizing Effects of Fissure Sealants Containing Bioactive Glass

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Objectives The aim of this study was to evaluate and compare the effect of commercial fissure sealants modified with bioactive glass (BAG) particles of different sizes (nano, micro, hybrid) on the demineralization resistance of the enamel and its contribution to the remineralization.

Methods Forty-five caries-free permanent molars were separated from their roots and embedded in epoxy resin. Then they were randomly divided into 5 groups and artificial initial caries lesions were formed on the occlusal surfaces of the teeth with a demineralization solution. After demineralization, the hardness and elasticity modulus values of the samples were measured by nanoindentation analysis. Commercial fissure sealant (Helioseal F Plus (HFP), Ivoclar,Vivadent) was applied to the control group. The experimental fissure sealants were applied to 3 groups: MICRO-BAG (15 wt% of micro-sized-BAG+ HFP), NANO-BAG (15 wt% of nano-sized-BAG+ HFP), and HYBRID-BAG (a combination of micro- (7.5 wt%) and nano-sized (7.5 wt%) BAG+ HFP). Fissure sealants were not applied to the negative control group. A second nanoindentation analysis was performed after a 7-day pH cycle. Then % surface hardness recovery values were calculated. Data were analyzed using one-way ANOVA, and t-test at an overall level of significance of 5%.

Results In all groups, the average nanohardness values after the pH cycle were higher than nanohardness values after demineralization. However, the difference was statistically significant only in the NANO-BAG group (p=0.036). The highest average surface hardness recovery value was in the MICRO-BAG group, followed by; HYBRID-BAG, control, NANO-BAG, and negative control groups. But there was no statistically significant difference between the groups (p=0.820). There was no statistically significant difference between the elastic modulus values after demineralization and after the pH cycle in all groups (p>0.05).

Conclusions Nano-sized BAG particles added to fissure sealants increase the resistance of the enamel tissue adjacent to the fissure sealant against demineralization and significantly contribute to its remineralization.
Qualification of Ion-Exchanges With Bioactive GIC Restoration on Root Caries
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Objectives
The objective of this study is to quantify the increased/decreased mineral concentration of root caries following the application of different restorative materials using non-destructive high-contrast X-ray microtomography (XMT).

Methods
A total of 93 extracted teeth with leathery root caries in severity indices 2 (cavitated) and 3 were assessed by the employment of clinical assessments, severity index and XMT. Six teeth were selected according to inclusion and exclusion criteria. Each lesion was subsequently cleaned and polished with a non-fluoridated prophylaxis paste (NUPRO Dentsply, USA). Visual-tactile examinations were carried out to in relation to hardness, cavitation, texture, size and severity index of root caries in addition to the baseline XMT scans (5 hrs). Root carious lesions were then restored using two different GIC materials without removing the leathery carious lesions: bioactive GIC material (Caredyne Restore) and GIC material (Fuji VII). The teeth in the control group had toothbrushing alone using toothpaste with 1,450 ppm fluoride. These samples were immediately scanned and a further scan was performed after three weeks. Each tooth was placed in artificial saliva (pH: 7) during this period and brushed twice a day using fluoridated (1,450 ppm) toothpaste. The time taken for each XMT scan was approximately 24 hrs. The XMT images were reconstructed for each tooth to measure the loss/gain at the demineralised sites.

Results
The XMT subtracted images clearly verified an increase in the Linear Attenuation Coefficient (LAC) within the leathery type carious dentine over three weeks. The bioactive glass ionomer cement showed significantly increased changes in LAC in comparison to the Fuji VII and control groups. Evidence of increased remineralisation and decreased demineralisation was observed with all groups.

Conclusions
This laboratory-based study demonstrated that high-contrast XMT is a useful apparatus to quantify the mineral loss/gain both from the GIC materials and root dentine. GIC materials, especially with the bioactive ingredient, used on cavitated root caries with severity indices 2 and 3 might have the potential to release ions in the demineralised dentine and promote remineralisation without the removal of carious lesions.
"Inflammageing” on Human Oral Cells: Correlation With Age-Related Diseases?

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Objectives Tissue inflammation has the potential to provoke cellular senescence, a process known as “inflammageing”, driving living cells to a state of permanent cell cycle arrest due to chronic antigenic irritation. This in vitro study aimed to shed light on the mechanisms of “inflammageing” on human oral cells.

Methods Primary cultures of human gingival fibroblasts (hGFs) were exposed to pro-inflammatory stimuli, including lipopolysaccharide (LPS), Tumor Necrosis Factor-alpha (TNFa), and gingival crevicular fluid (GCF) collected from active periodontal pockets of systemically healthy patients. Inflammageing was studied through two experimental models, employing either “aged” hGFs after prolonged passaging (p.10) that were exposed to the pro-inflammatory stimuli or young hGFs long-term exposed to the above stimuli from early- (p.10) to late-(p.10) passaging. Cells were evaluated for the expression of beta-galactosidase (histochemical staining) expression, senescence-associated genes (qPCR analysis), and for Senescence Associated Secretory Phenotype (SASP) biomarkers (proteomic quantitation analysis followed by bioinformatics analysis).

Results Significant increase (p<0.05) of beta-galactosidase positive cells was observed after exposure to each of the pro-inflammatory stimuli. The senescence-associated genes profile included upregulation for CCND1 and downregulation for C2CD5, SUSD6 and STAG1, which is typical for cellular senescence. Overall, pro-inflammatory licensing of late-passage cells caused more pronounced effects compared to long-term exposure of early-passage cells to the stimuli. The proteomic analysis showed induction of SASP evidenced by upregulation of several pro-inflammatory proteins (e.g. IL-6, IL-10, IL-16, IP-10, MCP-1, MCP-2, M-CSF, MIP-1a, MIP-1b, TNFb, sTNF-R, sTNF-R, TIMP-2) implicated in cellular aging and immune responses. The smallest impact on SASP was provoked by LPS and the most pronounced by GCF.

Conclusions This study demonstrated that long-term exposure of hGFs to various pro-inflammatory signals induced and accelerated cellular senescence with the most significant effects noted for the aged cells.

Periapical and Endodontic Status Among 65-Year-Olds in Oslo, Norway


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Objectives This study aimed to estimate the prevalence of apical periodontitis (AP) and root-filled teeth in the young-elderly population in Oslo, Norway, and to investigate associations of pathosis and treatment with selected socioeconomic and behavioral factors.

Methods A random sample of 450 65-year-olds living in Oslo, answered a questionnaire and underwent a clinical and radiographic examination (52% men and 48% women; response rate 58%). Based on detection from panoramic radiographs, periapical radiographs were taken of all root-filled teeth and of teeth with apical radiolucency, and their periapical status was evaluated using the Periapical Index. The dependent variables were presence of ‘untreated AP’, ‘root-filled tooth’, and ‘root-filled tooth with AP’ on individual level. The independent variables were gender, country of birth (western/non-western), education level (basic/higher), dental attendance pattern (regular/irregular), and smoking (never/former/current). The level of significance was set to p < 0.05 for statistical tests of associations.

Results The mean number of remaining teeth was 26 (SD: 4). AP was present in 45% of the individuals. Sixteen percent of the individuals had untreated AP and 38% had at least one root-filled tooth with AP. Sixty-six percent of the individuals had one or more root-filled teeth. Root-filled teeth were significantly more prevalent among regular dental attenders (68%) compared to irregular attenders (49%), and among former smokers (74%) compared to never-smokers (59%) (Chi-square test). In the logistic regression analysis of untreated AP, including all the independent variables, only current smoking was statistically significant (OR (95% CI): 2.9 (1.4-6.2), reference group: never-smokers).

Conclusions AP and root-filled teeth were prevalent in the present young-elderly population. The remaining number of teeth was high and a notable amount of untreated AP was observed, especially in smokers. The findings in the present young-elderly population indicate a substantial need for dental care associated with endodontic conditions in the future elderly.
**Effects of Experimental Nanohydroxyapatite and Er:YAG Laser on Dentin Hypersensitivity**

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**Objectives** This study aimed to evaluate the effects of experimental nanohydroxyapatite and Er:YAG laser on surface roughness and morphology for dentin hypersensitivity.

**Methods** Dentin discs (2mm thickness) were prepared from 50 human molars and randomly divided into 5 groups (n=10): Group 1: intact dentin (positive control), Group 2: demineralized dentin (negative control), Group 3: Bifluorid 10, Group 4: Er:YAG laser (50mJ,0.50 W, 10 Hz) Group 5: Experimental nanohydroxyapatite. Dentin hypersensitivity was simulated by 35% phosphoric acid for 1 min (except Group I). Surface roughness (Ra, mm) was measured by contact profilometry (Mahr, Marsurf PS1). Surface morphology and elemental content were evaluated by scanning electron microscopy (SEM) (Thermo Fisher Scientific, Phenom XL) and energy dispersive x-ray spectroscopy (EDS). The data were statistically analyzed using Welsch ANOVA and Games Howell. (p<0.05).

**Results** Group 4 showed significantly lower surface roughness than Group 5 and Group 3 (p<0.05). Besides, no significant differences in surface roughness were found among other tested groups. (p>0.05). SEM analysis indicated that most of the dentinal tubules were obliterated for Group 5. Besides, precipitant plugs with a few partially occluded dentinal tubules were observed for Group 3 while either decreased diameter of tubules or completely occluded tubules with melting appearance were detected for Group 4. EDS analysis revealed that Ca, P and O were detected for Group 5.

**Conclusions** Er:YAG laser treatment led to lower surface roughness than other treatment methods. The efficiency of nanohydroxyapatite and laser were sufficient on the dentinal tubule occlusion.

nanohydroxyapatite
Er:YAG Laser

Bifluorid 10
Occlusion: a key Parameter in the Construction of Patient-Specific Models

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Objectives Understand the mechanical behaviour of teeth is decisive to improve the long-term survival of our treatments. This has traditionally been analysed using finite element models and more recently by patient-specific modelling to consider individual variability and predict more precisely outcomes. Following the growth in computer science, heterogenous parameters have been used to develop these predictive models however without clear evidence on the exact influence of each parameter value and potential clinical implications. The aim of this study was to compare the influence of major simulation parameters on stress distribution using sensitivity analysis.

Methods The finite element model of a maxillary arch was constructed using a previously reported protocol. Following a full factorial design of experiments, sixteen four models were then generated to evaluate all combinations of the six following parameters axis of loading (axial/lateral force), intensity of loading (150/300 N), dentin elasticity (15/18.6 GPa), length of arch (3 teeth/ half full arch) element size (100/300 micron) ligament (absence/presence). For each model, the root von Mises stresses of the right first premolar were extracted and analysed using analysis of variance (ANOVA).

Results The stress distribution was similar for all models except for models with lateral force that present highest stresses on the peripheral parts of the root. Results of ANOVA analysis revealed that axis and intensity of loading were the most influent factors, explaining 40 % of the mechanical values.

Conclusions Define precisely the occlusion of the patient appeared essential to construct a predictive model as a small error on this parameter could greatly influence the biomechanical analysis and potentially lead to therapeutical choices poorly adapted to the patient. However, further investigations are required to evaluate if such an influence is present independently from other clinical parameters such tooth type or substance loss.

Artificial Intelligence for the Prediction of Oral Health From Breath

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Objectives Currently, diagnosis of oral disease (OD) relies on traditional clinical assessments. Yet, surfacing intelligent technologies show encouraging diagnostic alternatives. Recent publications have reported emanated volatile organic compounds (VOCs) in early stages of disease from related cells and bacteria. Early detection of pathogenic activity surrounding teeth/dental implants prevents irreversible damage. We hypothesize that each OD is associated with distinct VOCs, nanosensor (NS) detection of VOCs from exhaled breath (EB) employing machine learning algorithms offers great diagnostic potential. Our objectives are to identify oral disease-specific VOC profiles from EB by training, testing and cross-validating an Artificial-Intelligence based chair-side diagnostic device in a large patient cohort.

Methods Systematically healthy patients (n=300, >18 y.o) were phenotypically divided into 5 patient groups: 1. healthy periodontium (non-implant group) 2. healthy implants 3. periodontitis 4. peri-implantitis and 5.caries. EB were sampled and VOCs identified by gas chromatography-mass spectrometry (GC-MS) and Electronic nose. Finally, a chair-side Sniffphone device was trained and tested for disease prediction models.

Results Preliminary results (n=121) for GC-MS analysis yielded 18 significant VOCs following group comparisons. Discriminant Functional Analysis of a laboratory-based NS cross-reactive response to the collective VOCs enabled disease separation with 89.75-100% accuracy using up to 5 feature extractions.

Conclusions The future of dentistry lies in bridging the gap between technological innovation and oral healthcare needs. Our preliminary data strongly suggest that volatile profiling is achievable, and we believe that an AI-based chair-side device can improve early diagnostic accuracy, cut costs and mitigate oral disease outcomes.
Annotation Accuracy and Calculus Detection on Radiographs Using Deep Learning
Martha Duchrau, Joachim Krois, Falk Schwendicke

Objectives Labeling of medical data for developing deep learning-based software is time consuming and cost intensive, and the more exact labels need to be, the more efforts will be required for labeling. This becomes even more challenging when the data needs to be labeled by multiple experts. We aimed to assess the impact of annotation accuracy on the performance of a deep learning object detection model. Our case focused on the detection of dental calculus on bitewing radiographs.

Methods In total 6,840 bitewing radiographs had been labeled for dental calculus by 2 annotators using bounding boxes (BBs). To assess the impact of annotation accuracy, we simulated the behavior of three hypothetical experts, by dividing the dataset into thirds. For the first third of the dataset, we kept the original BBs to represent an accurate annotator. For the second and third third, we increased and decreased BB respectively, to simulate too small and too large labeling. In- and decreases were done to varying degrees, 25% and 50% relative to the original BB area. In a second experiment all BBs in the dataset were increased and decreased by 50%, respectively. For each experiment, an object detection model, YoloV5, was trained up to 300 epochs. Performance was evaluated using mean average precision with an intersection over union threshold of 50% (mAP50). A train/validation/test split of 80%/10%/10% was used.

Results The model trained on the original dataset achieved a mAP50 of 0.77, showing the feasibility of detecting dental calculus. Simulating different annotators with a deviation of 25% and 50% yielded mAP50 of 0.70 and 0.14, respectively. Increasing and decreasing all BBs by 50% resulted in mAP50 of 0.82 and 0.69, respectively.

Conclusions Consistently smaller or larger BBs for labeling did only limitedly affect model performance, while inconsistent BB sizes had significant negative impact. Calibration of annotators is highly important.

DMFT Index Evaluation Using Deep Learning on Panoramic Radiographs
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Objectives To evaluate the accuracy of a deep machine learning algorithm for assessing a DMFT index in panoramic radiographs of permanent dentition.

Methods The dataset consisting of 2500 depersonalized panoramic radiographs were assessed and Decayed-(DT), Missing-(MT), Filled-(FT) and Unerupted-teeth (UT) [PR1] were marked independently by two trained and calibrated examiners. The intra-observer reliability Kappa values were 0.972 and 0.944 and the inter-observer reliability - 0.894. Inconsistencies were corrected by a third examiner. Python 3.8 programming language was used to develop machine learning algorithm to recognize DT, MT, FT and UT. EfficientNet B7 algorithm was applied to learn and evaluate DMFT values in panoramic radiographs. The performance of the model was evaluated using F1-score metrics and tested on unseen 257 images. The accuracy of DMFT score was assessed with the mean absolute error (MAE), additionally the area under the receiver operating characteristic curve (AUC) was determined and the F1 score was included. For each data item examined, one of the following values was assigned: true positive/negative (TP and TN) and false positive/negative (FP and FN).

Results The number of TP values was 7658 (18.62%); TN - 32420 (78.84%); FP - 476 (1.16%); and FN - 566 (1.37%). The proposed method achieved a sensitivity of 0.9300, specificity of 0.9900, AUC of 0.9886, F-measure of 0.9360 and DMFT index MAE of 0.5136.

Conclusions The current machine learning algorithm showed promising results for an automatic DMFT index assessment in panoramic radiographs. More data is needed for the further development of machine learning algorithm.
Individualized Application of AI Combined With Micro-Invasive Caries Treatment was Cost-Effective
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Objectives There is uncertainty around the cost-effectiveness of artificial intelligence (AI) for medical applications. We assessed the value of information towards relevant input parameters (like AI accuracy, costs, population caries risk profile).

Methods The health intervention (AI) was a trained Convolutional Neural Network to classify bitewing radiographs. We employed a Markov model and microsimulation to quantify the value of information in a mixed public-private-payer perspective in German healthcare. Our health outcome was tooth retention years.

Results In the base-case scenario (uncertain accuracy, uncertain risk profile of the population, uncertain costs of the AI), AI was more effective (tooth retention for a mean [2.5-97.5%] 62.8 [59.2-65.5] years) and less costly (378 [284-499] Euro) than dentists without AI (60.4 [55.8-64.4] years; 419 [270-593] Euro). The economic value of reducing the uncertainty around the AI’s accuracy or costs was limited, while information on the risk profile of the population was more relevant. In high-risk populations, using an AI followed by micro-invasive therapy (e.g., resin infiltration) was highly cost-effective.

Conclusions Individualized application of AI for caries detection may optimize cost-effectiveness.

Local Delivery to Modulate Host Response as Potential Periodontitis Treatment.
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Objectives Periodontitis is characterized by the progressive destruction of tooth-supporting alveolar bone and gingival soft tissues, which are mainly caused by chronic inflammation in response to persistent bacterial insult. Recently it has become clear that the pathogenesis of periodontitis is associated with the high ratio of pro-inflammatory M1 to anti-inflammatory M2 macrophages. The goal of this study was to promote chemotaxis of M0 (immature) or M2 (anti-inflammatory) macrophages to the inflamed site and induce M2 phenotype polarization locally. We used C-C motif chemokine ligand 2 (CCL2) known to recruit M0 macrophages, and induce differentiation of endogenous M2 macrophages.

Methods We fabricated CCL2 controlled release microparticles (MPs) using poly (lactic-co-glycolic) acid. In vitro assessment for CCL2 release was performed and also the chemotaxis ability of CCL2. In addition, the effect of CCL2 delivery was assessed in vivo in three different ligature induced murine periodontitis models representing different clinical scenarios. We have used microCT analysis to determine alveolar bone loss, qPCR to detect inflammatory and reparative markers, TRAP, cell sorting and FACS analysis.

Results We have successfully synthesized CCL2-loaded PLGA microparticles (MPs) and quantified its sustained release. Microcomputed tomography showed in mouse ligature models a significant reduction of alveolar bone loss in the CCL2 MP group when compared to a blank MP group and to a no treatment group. Tartrate-resistant acidic phosphatase (TRAP) staining showed significantly fewer osteoclasts in the alveolar bone area of animals in the CCL2 MP group in alveolar bone area. Quantitative polymerase chain reaction (qPCR) in the ligature model, and showed a significant decrease of inflammatory markers as well as nuclear factor kappa-B ligand (RANKL) mRNA expression in CCL2 MP group.

Conclusions Manipulation of endogenous M2-phenotype macrophages using CCL2 MPs decreased the M1-phenotype/M2-phenotype ratio and prevented alveolar bone loss in mouse periodontitis models. The delivery of CCL2 MPs provides a novel approach to treat periodontal disease.
Age Related Attenuation of Apical-Periodontitis Development in Mice and Humans

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Objectives Older age is associated with reduced immune function. Our aim was to study how age affects the development of Apical periodontitis (AP), an inflammation occurring in the periodontal tissues surrounding the apices of infected teeth.

Methods AP was induced in mice, and 2 age groups were compared (young: 6-8 weeks, adult: 8-10 months). The teeth and jaws were histologically processed and stained by Hematoxylin Eosin (H&E), Brown and Brenn (B&B), and tartrate-resistant acid phosphatase (TRAP). In addition, the volume of the periapical (PA) lesions was evaluated by micro-computerized tomography (micro-CT). Cell density in the PA region was computationally assessed on H&E slides, and immune cell populations from the lesion were characterized by flow cytometry and immunofluorescence. Moreover, the progression of apical periodontitis in human patients (ages 8-98) was evaluated by comparison of x-rays in two different time points.

Results There was no difference in bacteria location in the canals between the groups. The periapical cell density was higher in the young group compared to the adults while the dominant immune cells in the lesions were neutrophils. All leukocyte types tested were present in higher numbers in the lesions of the young mice compared to adults, although the neutrophil presented the highest young/adult ratio. Immunofluorescence demonstrated the location of neutrophils in the lesion. More multinucleated osteoclasts were present in the lesions of the young mice, in correlation to the higher volume of bone resorption in the lesions of the young group. In human patients, the growth percent of PA lesions over time was attenuated in elder patients.

Conclusions Overall, we conclude that the immune reaction to AP stimuli was attenuated in the adult mice and human patients compared to young, perhaps due to immunological age-related changes.

Correlation Between Clinical Variables and Subgingival Microbiota

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Objectives The use of high-throughput sequencing has offered scientists a more complex and realistic picture of the composition of the subgingival microbiota. Such advancements might push periodontal medicine to a more precise, personalized and predictive era. Merging periodontal clinical variables (PCV) with other demographic and systemic clinical variables might allow to the development of predictive tools of the microbiome composition. The aim of this study was to study the correlation between the PCV and the microbial composition obtained by high-throughput sequencing.

Methods Subgingival samples were taken from periodontally healthy subjects (HS, n = 56) and periodontitis patients (PP, n = 121). Age, gender, bleeding on probing, clinical attachment loss and periodontal probing depth were recorded by the clinicians. Microbial composition was assessed through high-throughput sequencing of the V3-V4 regions of the 16S rRNA gene. Sequence analysis was performed following the DADA2 pipeline, and alpha and beta diversity were explored with the phyloseq R package. Associations between the studied variables were analysed with non-parametrical statistical tests, lineal models and correlation tests.

Results Significant differences were observed regarding the PCV of the samples between HS and PP. PCV showed little impact regarding the alpha diversity of the subgingival microbiota, except for the gender in HS, which showed higher richness (p = 0.04) and diversity (p = 0.043) in females. On the other hand, the gender in PP (p = 0.047) and the diagnose (p = 0.001) were able to significantly explain differences in the beta diversity. Significant differences were also observed regarding the differential abundance of certain genera.

Conclusions This study suggests that the association of PCV with the composition of the subgingival microbiota is not strong enough to allow the creation of predictive models. This model might be accomplished by broadening the array of variables and using identification techniques with higher resolution.
A Large-Scale Meta-Omics Analysis of Plaque Microbiome for Periodontal Diagnosis

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Objectives To analyse the predictive capacity of supragingival and subgingival plaque microbiota at the ASV-level to discriminate different periodontal health conditions (periodontal health, gingivitis, and untreated and treated periodontitis).

Methods A total of 120 patients (55 controls, 65 periodontitis) were selected for subgingival plaque collection in our setting. Sequencing of the V3-V4 16S rRNA gene region was performed in Illumina MiSeq. The obtained sequences and metadata were uploaded to the sequence read archive (SRA). Searches were performed in PubMed, Scopus, Embase, and the SRA to identify previously published Illumina V3-V4 sequencing studies on the supragingival and subgingival plaque microbiome in distinct periodontal conditions. Research that met the criteria for sequences and metadata were included in the meta-omics analysis, comprising a total of 2045 samples. Sequences were processed under the same bioinformatics protocol, which included the ASV-level classification and the use of an oral-specific database for taxonomic classification. The statistical analysis was conducted using the microbiome and mixOmics packages.

Results The predictive models for supragingival microbiota had AUC values ranging from 0.818 to 0.996, representing, at most, 2.39% of the ASVs and 17.37% of the species detected, respectively. Conversely, the predictive models for subgingival microbiota had AUC values ranging from 0.796 to 0.902, representing 2.85% of the ASVs and 21.67% of the species, respectively.

Conclusions Supragingival plaque microbiome was a better bacterial biomarker than the subgingival microbiome for discriminating periodontal health from untreated and treated periodontitis. The main health-predictor ASVs in both supragingival and subgingival plaque were: Rothia dentocariosa ASV2, Haemophilus parainfluenzae ASV3, ASV78, ASV45, and ASV46, Kingella oralis ASV66, Streptococcus vestibularis ASV27, and Actinomyces HMT 170 ASV119. The main periodontitis-predictor ASVs in dental plaque were: Tannerella forsythia ASV15, Filifactor alocis ASV19, Treponema denticola ASV38 and ASV150, Fretibacterium fastidiosum ASV97, Peptostreptococcaceae [XII][G-4] HMT369 ASV124, Streptococcus anginosus ASV142 and Peptostreptococcaceae [XII][G-6] nodatum ASV189.
The Salivary Microbiome for Discriminating Periodontal Conditions: a Meta-Omics Analysis
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Objectives To evaluate the predictive ability of the salivary microbiota at the ASV-level to distinguish distinct periodontal health conditions: health, gingivitis, and untreated periodontitis.

Methods One hundred and twenty-four patients (50 controls, 74 periodontitis) were selected for the collection of unstimulated saliva samples in our clinical setting. Sequencing of the V3-V4 16S rRNA gene region was performed in Illumina MiSeq, and the obtained sequences and metadata were uploaded to the sequence read archive (SRA). Searches were performed in PubMed, Scopus, Embase, and the SRA to identify previously published Illumina V3-V4 sequencing studies on the salivary microbiome in different periodontal conditions. Investigations that met the sequence and metadata quality criteria were included in the meta-omics analysis, comprising a total of 814 samples. Sequences were processed under the same bioinformatics protocol, which included the ASV-level classification and the use of an oral-specific database for taxonomic assignment. The statistical analysis was conducted using the microbiome and mixOmics packages.

Results The predictive models for differentiating periodontal health from gingivitis and periodontitis showed AUC values of 0.925 and 0.946, respectively; and required low numbers of the detected ASVs and species (0.15% and 1.16%, and 0.46% and 3.47%, respectively). In contrast, the model to distinguish gingivitis from periodontitis required more taxa (1.29% of ASVs and 10.69% of species), with AUC values of 0.999.

Conclusions The salivary microbiome presented an outstanding ability to discriminate between periodontal health, gingivitis, and untreated periodontitis. The main health-predictor ASVs in saliva were: Streptococcus sanguinis ASV228 and ASV619, and Rothia aeria ASV507. Conversely, for gingivitis they were: Prevotella melaninogenica ASV7, Veillonella rogosae ASV72 and ASV241, and Veillonella atypica ASV3401; and for periodontitis: Sacchari BTASV095869 ASV36 and Leptotrichia sp. HMT417 ASV33 as ASVs of higher abundance, Filifactor alocis ASV19, Tannerella forsythia ASV15, and Treponema denticola ASV38 as ASVs of lower abundance.
Parabiotic Generation Using Ultrasound From *L. rhamnosus* and *L. reuteri* Probiotics

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**Objectives** Although probiotics' use as alternative dental aids is increasing, the presence of live bacteria in these products has been a deterrent to their significant popularity in clinical practice. Administration of inactivated microorganisms, or parabiotics, has been tested in other fields to counteract this problem, but has only been sparingly tested in dentistry. This research aimed to assess the effects of ultrasound technology on commonly used probiotics *L. rhamnosus* and *L. reuteri*.

**Methods** *L. rhamnosus* GG (ATCC) and *L. reuteri* DSM 17938 (Biogaia Protectis®) were cultured in MRS at 37°C in 5% CO₂. Ultrasound treatment (UP400St, Hielscher) was carried out on 24-hour cultures (3 x 10⁸ CFU/mL) on single species and 1:1 mixes at 240 W, 4.5 cm probe immersion depth (S24d3 sonotrode, Hielscher) for 3, 6, 8, 10 and 15 minutes.

Viability assays (colony counts, BacLight® and MTT) confirmed viability following treatment. Structural changes post-treatment were investigated through Scanning Electron microscopy (SEM) (Zeiss, 8 KV) following glutaraldehyde fixing, osmium tetroxide staining, ethanol dehydration and gold plating, while growth inhibition was assessed (spot test) against *Streptococcus mutans* (NCTC 10449 and ATCC 25175), *Streptococcus gordonii* (DL1 and ATCC 10558) and *Streptococcus oralis* (ATCC 35037). Sterile-filtered supernatants were also analysed (Bradford's assay, Data-Independent Acquisition proteome analysis (BGI)).

**Results** All probiotic groups lost viability after 15 minutes of treatment (p < 0.001). SEM showed progressive treatment time-dependent collapse of the bacterial membrane. *L. rhamnosus* products showed greatest inhibition, albeit with a treatment time-dependent decrease. Decreased viability, altered bacterial membrane and decreased inhibition with increased treatment time suggested probiotic product secretion, confirmed through supernatant analysis.

**Conclusions** To our knowledge this is the first time parabiotics for oral health applications have been produced with the proposed methods. This research highlights ultrasound technology's potential for translation for use on other probiotics for a wide range of medical applications.
Impact of Long-Term Amoxicillin Treatment on the Gut Microbiome and Resistome

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Objectives The collateral impact of antibiotics on the microbiome has lately attained increasing attention. However, the knowledge of ecological consequences of long-term antibiotic exposure on the gut microbiome, including the development and persistence of antibiotic resistance, is still limited. Here, we aim to investigate the effects of long-term exposure of the most commonly used antibiotic (amoxicillin) on the gut microbiome and resistome.

Methods Fecal samples were collected from 20 patients receiving 100 days of amoxicillin treatment as part of a Norwegian multicenter clinical trial on chronic low back pain (AIM study). Samples were collected at baseline, last day of treatment, and 9 months after the administration of antibiotics or placebo. Whole metagenomic shotgun sequencing and functional metagenomics sequencing data were utilized to characterize the abundance and diversity of microbial and resistome composition.

Results While the microbiome profiles of placebo subjects were relatively stable over time, significant changes in the diversity and overall microbiome composition were observed upon amoxicillin treatment. In particular, health-associated short-chain fatty acids producing species significantly decreased in proportion. However, these changes were short-lived as the gut microbiota showed recovery to baseline levels 9 months post-treatment. On the other hand, exposure to long-term antibiotics was associated with an increase in total antimicrobial resistance load and diversity of antimicrobial resistance genes, which remained statistically significant even at 12 months compared to the placebo group. Additionally, these long-lasting changes were more targeted to the antibiotic treatment as the beta-lactamase resistance genes were significantly enriched in response to amoxicillin. Although the antibiotic altered the resistome composition, the impact of amoxicillin remained specific to individual subjects.

Conclusions Overall, our results suggested that the impact of prolonged amoxicillin exposure was more pronounced and long-lasting in the fecal resistome rather than in microbiome composition. Such information is relevant for designing rational administration guidelines for antibiotic therapies.

The Impact of Ampicillin on the Oral Microbiome and Resistome

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Objectives Antibiotic exposure can lead to major perturbations in the human microbiome. In addition to promote dysbiosis, antibiotics can select for antimicrobial-resistance. In the oral cavity, penicillin-type antibiotics are detected at low concentrations in saliva following systemic administration, and in vivo studies have reported changes in oral microbiome composition. Due to challenges in quantifying in vivo effects of antibiotics in complex microbial communities and the presence of confounding factors, reproducible laboratory models are widely used. Here, we aim to investigate the effect of low concentrations of a broad-spectrum antibiotic, ampicillin, on oral microbiome ecology.

Methods We utilized an ex vivo oral microbiome model with a highly complex microbial diversity. Pre-formed oral biofilms from three donors were exposed to ampicillin at three concentrations: 0.025, 0.05, and 0.1 µg/ml, with three replicates each, including a control without antibiotic exposure. We used whole metagenomics sequencing to characterize the microbiome taxonomic and resistance profiles.

Results 284 million paired reads were generated. Samples from the three donors showed significant inter-individual differences. Clustering analysis using multi-dimensional-scaling revealed a pattern in which antibiotic exposure resulted in higher dissimilarity in microbial composition across replicates, compared to the non-exposure controls. Low concentrations of ampicillin showed decrease in microbiome diversity, as revealed by comparisons at the genus and species levels. Shifts in specific bacterial species varied in an individual basis. For oral resistome, 28 different antibiotic resistance genes (ARGs) were found in the three donors. In all cases, oral resistome diversity showed no major changes following ampicillin treatment, although some ARGs were only detected in the ampicillin exposed samples.

Conclusions In conclusion, our results using a model-system for oral microbiomes show that short-term exposure of low concentrations of ampicillin can lead to ecological disturbances in microbial composition and resistance patterns, and support in vivo studies showing that ampicillin at low concentrations can promote dysbiosis.
Assessment of Early Orthodontic Treatment Need Indicators on Transylvanian Children
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Objectives Epidemiological studies represent a powerful tool for the early diagnosis of malocclusion and commencement of an orthodontic treatment. Our study aimed to assess the orthodontic treatment need in a pediatric sample from the Transylvanian area in order to emphasize the importance of oral health related preventive strategies.

Methods The study was conducted on 188 children between 5 and 6 years of age from nine schools and two kindergartens in the Transylvanian region of Romania. The study consisted of a brief clinical examination of children using, the Index of Orthodontic Treatment Need-Dental Health Component (IOTN-DHC), and a questionnaire referring to the child’s parental education, frequency, and motivation of visits to the dentist, dental care habits.

Results Among the most frequent malocclusions was the overbite (30.31%), followed by deep bite (19.14%) and crossbite (14.89%). The total prevalence of orthodontic treatment need was 37.23% while 63 % of the sample presented more than one altered occlusal parameter. No statistical differences were found between female and male patients but differences between urban and rural inhabitants were found and were somewhat explained also by the answers on the questioners.

Conclusions National oral health preventive strategies should include effective orthodontic prevention programs including pediatricians and school personnel training in this respect.

Biomimetic Calcium Phosphate Coating on Medical Grade Stainless Steel
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Objectives Stainless steel (SSL) minicrew implants have been applied in orthodontic clinics. They are of excellent physical properties but relatively poor biocompatibility. It is unknown whether the biocompatibility of medical grade smooth stainless steel can be improved by modifying SSL with a biomimetic calcium phosphate coating with or without additional drugs.

Methods Titanium (Ti) discs and SSL discs were used in this study. The immersion period in a biomimetic modified Tyrode solution (BMT) was 24h at 37 degree for Ti discs to form an amorphous layer. For SSL discs, the immersion periods were 0h, 12h, 24h, 36h and 48h respectively. After this procedure, the discs were immersed in a supersaturated calcium phosphate solution (CPS) for 48h at 37 degree to form a crystalline layer. Physical, chemical and biological properties of the BioCaP coating were analyzed and compared. In addition, to assess protein loading profile and surface wettability variations of the crystalline layer, bovine serum albumin (BSA) was incorporated as a model protein.

Results The amorphous and crystalline layers both increased the wettability of SSL. With the increase of immersing period in BMT, the average roughness of coating surface, the size of crystals, the amount of BSA incorporated, cell seeding efficiency and cell spreading area of the crystalline layer were significantly increased.

Conclusions These in vitro results show that the BioCaP coating can improve biocompatibility of the smooth medical grade SSL and serve as a carrier system for bioactive agents. In vivo study will be carried out by mimic the orthodontic treatments in the future.
**OO51**

**Periodontal Ligament Stem Cells Protected Alveolar Bone During Tooth Movement**

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**Objectives** To investigate whether the transplantation of human periodontal ligament stem cells (PDLSCs) can alter the rate of orthodontic tooth movement (OTM) and/or alveolar bone morphometry in a rat model.

**Methods** PDLSCs were isolated from extracted teeth, characterized as mesenchymal stem cells and labeled with gold nanoparticles for *in vivo* cell tracking. 12 twelve week-old male Wistar rats were divided into (1) transplantation and (2) control group, both of which were subjected to OTM by applying a constant orthodontic force of 25cN between one of the upper first molars and a mini-screw. In the transplantation group, 1x10⁶ labeled PDLSCs were embedded into Matrigel and transplanted via periodontal ligament injection. In the control group, a sham injection with only Matrigel was performed. *In vivo* micro-CTs were taken before and 31 days after orthodontic force application. OTM and bone morphometry were compared between both groups by two-way repeated measures ANOVA.

**Results** No significantly differences were observed in OTM rate between the transplantation and control groups. However, bone mineral density, bone volume fraction, trabecular thickness and trabecular number of the alveolar bone in the transplantation group were significantly higher than in the control group 31 days after force application (*P* < 0.05).

**Conclusions** Although the transplantation of periodontal ligament stem cells did not alter OTM rate, it might reduce bone loss and thus protect the alveolar bone during OTM.

**OO52**

**Genetic Variants of American-European Families With Class-III Phenotype: an Update**

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**Objectives** Considering that family-based studies are still one of the most effective tools to explore genetic regions and identify candidate genes phenotype-related to complex diseases, this research aims to report the latest findings of genetic linkage analysis associated to the skeletal Class III malocclusion (sCIIIIm) phenotype in European and American populations.

**Methods** A systematic literature search was conducted following the PECO question ‘what are the genetic variants linked in families with one or more members exhibiting sCIIIIm phenotype?’. Participants: affected family members of European or American ethnic origin, with absence of syndromic conditions or dentofacial trauma. Exposure: sCIIIIm phenotype clinically or radiographically diagnosed. Control: unaffected family members. Outcome: [Primary]: identification of candidate genetic variants, mutations or loci linked to the sCIIIIm phenotype; [secondary]: description of distribution according to ethnic origin, inheritance patterns and sex linkage.

**Results** After running the search string, duplicates removal, and screening by title and abstract a total of four articles met the criteria. Colombian, Brazilian, Estonian, and Italian multigenerational families were studied. 12q23, 12q22-q23, 10p12.1 loci were reported as carriers of candidate markers of interest: *IGF1, HOXC, COL2A1, DUSP6* (c.545C>T; p.Ser182Phe), and *ARHGAP21* (Gly1121Ser). Only two articles found variants of interest on the same locus (12q23), and both described a wide characterization of the sCIIIIm phenotype; mandibular, maxillary or mixed origin phenotypes. All the articles reported an autosomal dominant inheritance pattern with incomplete penetrance, and no differences between sex.

**Conclusions** Family-based studies on sCIIIIm phenotype, disclose locus 12q23 as carrier of variants of interest shared by Estonian and Colombian families; these genotype variants respond to a wide phenotype characterization of sCIIIIm; an autosomal dominant inheritance mode and no sex linkage is exhibited.
Age-Related Changes in Facial Soft Tissues and Muscle Pressure.

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Objectives Aging of facial soft tissues can influence extraoral muscular pressure on the dentoalveolar structures and therefore lead to malocclusion or post-orthodontic treatment relapse. This study aims to analyse the effect of age on 3D-facial soft-tissues, as well as on lip and cheek pressure in a sample of subjects with normal occlusion.

Methods 120 orthodontically untreated subjects with normal occlusion were divided into three groups: children (with mixed dentition), adolescents (with permanent dentition and until 17 years of age) and adults (18 to 55 years). 3D-facial images were captured using the Vectra H1 system, while lip and cheek pressure was recorded using the IOPI instrument. 3D-Images were prepared using MeshLab, MeVisLab and processed using the Meshmonk tool. Partial Least Square (PLS) regression and two-way ANOVA were used to analyse the age- and sex specific differences.

Results Significant facial changes were observed between all age groups. Compared to children, adult males present marked retrusion of the cheeks while the points along the facial midline are directed outwards, while adult women present more protruded nasal bridge and tip, subnasale, supraorbital area and cheeks, while the upper, lower lip and upper forehead are more retruded. Lip pressure was lower in children compared to adolescents and adults (2.0 and 3.42KPa respectively). Right and left cheek pressure was significantly higher in adults than in children and adolescents (6.83KPa, 5.17KPa, and 7.23KPa, 5.37KPa, respectively).

Conclusions Facial soft tissue morphology changes with age and correlates with increasing lip and cheek pressure. Clinicians should consider these changes during orthodontic diagnosis and treatment planning by incorporating non-invasive modalities such as 3D images and measurement of oral muscle pressure in routine orthodontic practice. These ‘average templates’ of normal occlusion faces could be used to determine how much do patients diverge from the norm and educate the need for long-term orthodontic retention.

The Molecular Mechanism Underlying Orthodontic Relapse

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Objectives Orthodontic tooth relapse (OTR) is an undesirable outcome of orthodontic treatment. Recent studies demonstrated the crucial role of the immune system in orthodontic tooth movement (OTM). However, whether this mechanism regulates OTR is still unclear. Here, we aimed to investigate the role of the innate and adaptive immune response during OTR using in vivo time-course gene expression profiling.

Methods The OTR model included two steps: first, initiating OTM and then allowing the mice’s teeth to relapse. To initiate OTM, Ni-Ti springs were set between upper left first molars and upper incisors of C57BL/6 mice. Following 14 days, springs were removed and the molars were allowed to relapse for 0,1,7 days. To validate the OTR model, we measured relapse rate by Micro-CT (n=6\group). To investigate gene expression profiles, the periodontal tissue blocks were extracted and RNA sequencing was performed (n=5\group). Gene function was inferred by Gene Set Enrichment Analysis (GSEA). The K-means algorithm and pathway analysis method were used to identify gene clusters in a time-course manner. qRT-PCR was used to validate genes of interest (n=7\group).

Results Micro-CT analysis showed a OTR rate of 50%,57%,59.9% following 1,7 and 14 days, respectively, compared to 0% in the control group (0-day relapse). A total of 605 differentially expressed genes (DEGs) were identified over the OTR period with the highest number (514) at day-7. GSEA in day-1 showed an upregulation of genes related to collagen biosynthesis, cartilage development, osteoclast differentiation, angiogenesis, inflammatory response, and TNF-α signaling. Day-7 showed an upregulation in genes related to the innate-adaptive immune system, antigen processing, and cytokine signaling. qRT-PCR results showed that over the relapse period there was an upregulation in the innate and adaptive immune cells such as neutrophils, macrophages, T cells, and B cells validating RNA sequencing results.

Conclusions This is the first in vivo report of gene expression signatures of OTR in a time-dependent manner. The gene sets found constitute attractive targets for future molecular basic science studies in the orthodontic field and will contribute to finding novel therapeutic means to clinically control it.
The Unrecognized Mechanism of B-Cells Activation in Response to Orthodontic Forces

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Objectives Orthodontic-tooth-movement (OTM) is achieved by bone-remodeling (BR) mediated via an aseptic-inflammatory process. B-cells, which play a crucial role in the etiology of inflammatory bone-diseases, are activated by antigen-recognition. Yet, whether mechanical-forces can activate B-cells remains unknown. Here, we aim to investigate the role of B-cells and their mechanism-of-action (MOA) in OTM.

Methods NiTi-springs ligated between maxillary-first-molars (M1) and incisors in CD19cre homozygote and WT (C57BL/6) mice, generating mesial movement of M1. To study whether force application activates B-cells, total RNAs were extracted from dissected tissue blocks after 1, 3, 7 and 14 days, and exclusive B-cells genes were measured by qRT-PCR and immunofluorescence (IF) staining. OTM-distance and bone parameters were measured by μCT and histological-staining. To investigate B-cells’ MOA, RNA-sequencing was performed after 3 days of OTM, and validated by qRT-PCR. Additionally, an OTM re-activation model was established to study whether B-cells can be primed in response to orthodontic-reactivation-forces.

Results B-cells genes expression (CD19, B220 and CD79a) peaked at day 3 following OTM. IF demonstrated CD19B-cells in the alveolar bone, which were clustered in the compressed PDL at day 14. After 14 days, OTM rate was significantly decreased in CD19cre mice compared to WT control with significant reduction in osteoclasts number and bone formation process. RNA sequencing revealed 272 differentially expressed genes in CD19cre mice following 3 days, exhibited a distinct downregulation of genes associated with bone formation (Amelx), pro-inflammatory chemokines which promote bone resorption (CxcL 2/3/5, Ccr6), B-cells homing chemokine (CxcL13) and genes associated with negative regulation of bone formation (Bglap2/3). In the re-activation OTM, acceleration of the OTM-rate was significantly impaired in the CD19cre mice compared to the WT mice.

Conclusions This study demonstrates a previously unrecognized mechanism that B cells are activated in response to orthodontic-forces and required for BR and OTM via their CD19 receptor activation followed by unique cytokines/chemokines expression. Moreover, we highlighted the possibility of B-cells priming due to mechanical-forces which emphasize the adaptive immunity contribution in OTM.
3-Year-Survival of CAD/CAM Resin-Based Composite Restorations in Severe Tooth-Wear Patients

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Objectives The aim of this prospective study was to assess the clinical performance of minimal-invasive CAD/CAM resin-based composite restorations in severe tooth-wear patients by evaluating their survival after 3-years.

Methods Twenty-two patients with generalized severe tooth wear with functional and/or esthetic problems were included in the study. Following minimal-invasive preparation, CAD/CAM resin-based composite restorations (LAVA Ultimate, 3M) were adhesively luted (ESPE Sil, Scotchbond Universal, RelyX Ultimate, all 3M). Patients were recalled after one month, one year and three years. Failures were categorized as F1 (severe deficiencies resulting in replacement/extraction), F2 (localized deficiencies resulting in re-cementation/repair) and F3 (small chippings resulting in refurbishment/monitoring). Survival was evaluated using lifetables and Kaplan-Meier graphs, distinguishing between failure categories and tooth type (front teeth, premolars, molars).

Results 568 indirect restorations in 21 patients (age 39.5±10.6y; 3 female/18 male) were evaluated for survival of restorations up to 3.5-years (one patient dropped out due to financial reasons). Until this time-point, a total of 42 failures were recorded (F1:4, F2:17, F3:21). Overall survival concerning F1 was 99.3%, for F1+F2 it was 96.0% and for F1+F2+F3 it was 91.5%. AFR’s were respectively 0.2%, 1.2% and 2.5%. Tooth type had an influence on survival probability, which was most distinct for F1+F2+F3: For front teeth survival was 98.8% (AFR=0.3%), for premolars it was 87.8% (AFR=3.6%) and for molars survival was 80.3% (AFR=6.1%).

Conclusions Minimal-invasive CAD/CAM resin-based composite restorations showed a good clinical mid-term survival for restorative rehabilitation of severely worn dentitions. Restorations on molars and premolars showed a higher failure probability than those on front teeth.

Three-Year Clinical Performance of a Universal Adhesive in non-Carious Cervical Lesions.

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Objectives The aim of this randomized controlled clinical trial was to evaluate the 3-year clinical performance of a universal adhesive (Clearfil Universal Bond Quick (CUBQ); Kuraray Noritake), using two different application modes (etch-and-rinse vs self-etch with prior selective enamel etching), when restoring non-carious cervical lesions (NCCLs).

Methods Fifty-one patients participated in this study. A total of 251 NCCLs were assigned to two groups: 1. CUBQ in the etch-and-rinse mode (n=122)(CUBQ, ER) and 2. CUBQ in a self-etch mode with prior selective etching of enamel with phosphoric acid (n=129)(CUPQ, SEE). The same resin composite Clearfil Majesty ES-2 (Kuraray Noritake) was used for all restorations. The restorations were evaluated at baseline, 1 and 3 years using FDI criteria: marginal staining, fracture and retention, marginal adaptation, postoperative sensitivity and recurrence of caries. Statistical analysis was performed using a logistic regression model with generalized estimating equations (2-way GEE model).

Results The patient recall rate at 3 years was 90%. At this recall, the overall success rate was 84% and 83 % for CUBQ-SEE and CUBQ-ER respectively. In total, 38 restorations (19 CUBQ-ER, 19 CUBQ-SEE) failed because of loss of retention, fracture, severe marginal defect and/or discoloration. A retention rate of 87% and 86% was recorded for CUBQ-ER and CUBQ-SEE, respectively. No significant difference was observed between the two groups for any of the evaluated parameters.

Conclusions After 3 years of clinical service, Clearfil Universal Bond Quick performed similar in an etch-and-rinse mode and in a self-etch mode with prior selective enamel etching.
20-Year Follow up of ‘Open Sandwich’ and Total-Etch Posterior Composite Restorations

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Objectives To compare clinical performance of resin composite posterior Class II restorations placed with either an etch-and-rinse adhesive or an open sandwich technique using glass-ionomer cement and to investigate proximal dissolution of GIC over time.

Methods Data on Class II restorations placed by one dutch GDP between 1990-2016 were collected from patient files, including caries risk, tooth related variables (nr. Surfaces, tooth number, presence of endo) and applied materials. Before 2001, the GDP placed posterior composite restorations routinely using the open sandwich technique with glass-ionomer cement and composite. After 2001, he switched over to total-etch technique using etch-and-rinse 3-step adhesive when placing a Class II composite restoration.

Results 675 Class II restorations were placed in 91 patients (age 15-79 years; 40m, 51f). 491 (73%) were total-etch restorations (observation time 2-18 years), and 184 (27%) open sandwich restorations (observation time 19-29 years).

AFRs were after 15 years 2.9% for total etch restorations and 9.7% for open sandwich restorations. secondary caries was equally distributed among the groups. 27% of the failures in the open sandwich group were due to proximal dissolution of glass ionomer cement.

The Cox-regression showed a significant higher risk for failure for the open sandwich technique compared to total-etch class II composite restorations (HR=2.9; p<0.001)

Conclusions On the long term, open sandwich restorations show inferior clinical performance compared to total-etch class II composite restorations. Proximal dissolution of GIC seems a clinical problem for open sandwich restorations.

Direct Class-v Restoration Survival, a Practice-Based Prospective Cohort Study

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Objectives To investigate clinical performance of resin composite Class-V restorations and involved operator, patient and restoration variables.

Methods From digital patient files, data on Class-V restorations placed in 10 practices by 20 dentists were collected, placed between January 2015 and June 2021. The following data were retrieved: Patient level: Gender, age, SES, general health score, periodontal condition, caries risk, presence of parafunctional habits. Tooth/restoration level: Tooth type, endodontically treated (y/n), reason for restoration placement, used restorative material and adhesive. The date of placement, date of last check up-visit and when applicable, date and type of intervention. Tooth extraction, endodontic treatment and placement of a crown or a new restoration including the same surfaces as the existing restoration, were considered as failure. Kaplan Meier statistics were applied and Annual Failures Rates (AFR) were calculated. To investigate influence of variables on longevity of restorations, a multilevel Cox-Regression was conducted (p<0.05), with clustering of data for restorations within the same patient.

Results AFRs were calculated after 5 years; overall 2.6%, 3.2% for restorations placed due to caries and 2.2% for restorations placed due to NCCL.

The Cox-regression showed a significant higher risk for failure for the Class-V restorations placed due to caries (HR=1.79; p=0.004). No significant effects were found between other patient-related-variables, operators and different composite and adhesive materials.

Conclusions Diagnosis for Class-V composite restoration placement has a significant effect on restoration survival. Restorations placed due to caries show inferior clinical performance compared to restorations placed due NCCL. Other operator, patient and material related variables play a minor role.
A Ten-Year Clinical Evaluation of Direct and Indirect Composite Restorations
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Objectives To assess the clinical efficacy of posterior composite resin restorations placed directly and indirectly in posterior teeth after 10 years.

Methods One hundred eight cavities in 54 patients were restored with three direct composite resins (Filtek SupremeXT (FSXT), Tetric Evo Ceram (TEC), Aelite Esthetic (AE)) and two indirect composite resins (Estenia(E) and Tescera ATL(TATL)). All restorations were evaluated by two examiners using the USPHS criteria, at baseline and 10 years after placement. Statistical analysis was completed with Fisher’s exact and McNemar Chi-square tests.

Results At baseline, the number of teeth restored was reduced by 12% (13 teeth) due to inaccessibility to patients or loss of teeth. Charlie score was recorded in all restorative systems in the 10-year evaluation of our study, which did not have a Charlie score in its 5-year evaluation. Only missing teeth made up the Charlie rating. Evaluations of all remaining restorations were recorded with Alpha and Bravo scoring. At the end of 10 years, Bravo scores were recorded for color match in all restorations. All evaluations of the remaining restorations in the TEC group, except the color match, were recorded with an Alpha score of 95%. Post-operative sensitivity was maintained with an Alpha score of 95% only in the TEC and AA groups. In terms of gingival adaptation and secondary caries, the remaining restorations in all groups except the TATL group were recorded with Alpha grading only. Surface texture, marginal integrity, marginal discoloration, and retention were recorded with Bravo scores at different percentages at the end of 10 years for all groups (except TEC).

Conclusions In the study, which was examined according to USPHS criteria, no statistically significant difference was found between direct and indirect composite resin restorations over a 10-year period. Restorations made in the light of the information obtained were clinically successful in the medium and long term.

Clinical Evaluation of Universal Bonding Agents: 3 Years Result
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Objectives The aim of this study was to evaluate the 3-year adhesive performance of class II restorations made using six different universal bonding systems in patients treated at Selcuk University Faculty of Dentistry Restorative Dentistry Clinic.

Methods In this study, restorations made with routinely used universal bonding agents (G-Premio Bond (GC Corporation, Japan)(15), Tetric N-Bond Universal (Ivoclar, Liechtenstein)(15), Clearfil Universal Bond (Kuraray, Japan)(21), All-Bond Universal (BISCO, USA)(17), Prime&Bond Universal (Dentsply, Sirona)(23), Nova Compo B Plus+ (Imicryl, Turkey)(16)) in patients aged 18-25 years who applied to our clinic due to class II caries were evaluated. While evaluating the restorations, an adhesive success table was created based on the Modified USPHS Criteria (marginal adaptation, marginal discoloration, surface texture, color change, marginal integrity, anatomical form and postoperative sensitivity).

Results According to the findings obtained from the restorations evaluated at the end of 3 years, postoperative sensitivity; Charlie (C) score was recorded according to Modified USPHS criteria in a single filling using G-Premio Bond (GC Corporation, Tokyo, Japan). Likewise, according to the secondary caries evaluations, a C score was observed in both G-Premio Bond (GC Corporation, Tokyo, Japan) and Prime&Bond Universal (Dentsply, Sirona) fillings. For retention; the only restoration in the Clearfil Universal Bond Group was determined as C. When other values were examined; restorations remained within acceptable limits with Alpha (A) and Bravo (B) scores.

Conclusions When the 3-year clinical performance of 107 restorations made with universal bonding agents was evaluated, 3 restorations were unsuccessful and a success rate of 97.2% was found. This success rate can be explained by the ionic binding of 10-MDP (methacryloxyloxydecyl dihydrogen phosphate) in new generation universal bonding agents to the calcium in the dentin(hydroxyapatite), through the hydrophilic group included in the molecule with a micromechanical bond.
3D-Inspired Bioengineered Tissue Flaps for Tissue Regeneration
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Objectives Functional regeneration of complex large-scaled defects requires heavily vascularized tissue grafts with patient-specific anatomy. Autologous tissue harvest, considered the standard of care in surgical settings, has considerable drawbacks such as tissue-site morbidity and post-operative pain. Tissue engineering approaches relying on bio-inspired additive manufacturing techniques and induction of vascular connectivity with hosts can promote graft sustainability while enabling proper adaptation within tissue defects during engraftment.

Methods Neo-tissue flaps developed via stem-cells loading onto biocompatible and biodegradable soft tissue scaffolds. Following in-vitro induction, bioengineered scaffolds are combined with a bone matrix to yield a composite neo-tissue. These, in turn, undergo axial vascularization in a rat arteriovenous model. Both ex vivo and in-vivo assessments of maturation and implantation are performed.

Results Pre-vascularization and osteogenic induction of tissue constructs are validated in vitro and in vivo, and high-resolution micro-computed tomography is employed to assess bone deposition, remodeling, and micro-vessels within neo-tissues.

Conclusions The presented methodology promotes neo-tissue creation for soft and hard tissue bridging. In addition, the underlying angiogenic connectivity between host and graft is evaluated, and upscaling of human-sized constructs is performed.

Role of Dental Pulp Stem Cell-Derived Neurotrophic Factors
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Objectives Dental pulp stem cells (DPSC) are increasingly considered an effective source to rescue and repair damaged neural cells. This study aimed to examine the role of specific neurotrophic factors in DPSC-promoted neuronal survival and neurite outgrowth length by applying specific neutralising antibodies. For this purpose, the established neuronal rat PC-12 cell line was used in comparison with primary trigeminal ganglion cells (TGNC).

Methods DPSC-conditioned medium (CM) was collected from 72h serum-free rat incisor-derived DPSC cultures. Neurotrophic factors (NGF, BDNF, NT-3, and GDNF) were analysed by specific ELISAs. Under the influence of specific neutralising antibodies against NGF, BDNF, GDNF, NT-3 or mixture of these antibodies (R&D systems), the cell survival and neurite outgrowth length of NeuN and MAP-2 positive PC-12 as well as TGNC were measured using Image J analysis. Gene expression levels of axonal growth-associated protein 43 (GAP-43), Synapsin-I, NeuN, MAP-2, βIII-tubulin and thermal receptor vanilloid potential-I (TRPV1) were analysed by RT-PCR.

Results The number of DPSC-promoted surviving PC-12 cells was markedly reduced by the addition of anti-GDNF antibodies while the neurite outgrowth was significantly attenuated by anti-NGF, anti-GDNF and anti-BDNF antibodies. In TGNC cultures, inhibition of NT-3 resulted in significant reduction of neuronal survival whereas neurite outgrowth was significantly attenuated by blockage of GDNF. Furthermore, neutralisation of GDNF and NGF resulted in marked downregulation in NeuN, MAP-2, βIII-tubulin and GAP-43 gene expression.

Conclusions GDNF proved to be critical for PC-12 cell survival, whilst NGF, BDNF and GDNF were involved in the stimulatory action on PC-12 neurite outgrowth. TGNC, however, were predominantly dependent on GDNF for their neurite outgrowth, but not so for cell survival. Thus, this study suggested that DPSC promoted survival and regeneration of primary TGNC and PC-12 neuronal cells by different neurotropic mechanisms.
**The Immunomodulatory Properties of Dental Pulp Stem Cells**

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**Objectives** Dental pulp is a cell rich connective tissue, where resident cells are programmed to perform specialised functions to maintain a homeostatic environment. Dental pulp contains several immunocompetent cells that orchestrate a protective response to injury or microbial invasion. Dental pulp stem cells (DPSCs) are specialized mesenchymal stem cells present in the dental pulp which are known to have high proliferative and differentiation capabilities. Recently, it has become apparent that these cells have multifunctional roles. Indeed, evidence suggests they also have intricate immunomodulatory properties.

**Methods** A holistic approach to uncover the immunomodulatory properties of DPSCs was undertaken using Nanostring™ technology. DPSCs were stimulated with Toll like receptor (TLR) ligands for 4 and 24 h and changes in levels of mRNA expression of 800 immunoregulator molecules determined using the Nanostring™ immunology panel. To validate the findings, changes in expression of key molecules of interest were also further investigated using qPCR, ELISA and immunocytochemistry. Type 1 Interferon signalling pathway was validated further using the Applied Biosystems™ TaqMan™ Array Human Interferon Pathway 96-well Plate arrays.

**Results** The data reveals that DPSCs show differential immunomodulatory properties in response to *E. coli* LPS (TLR-4 agonist), PAM2CSK4 (TLR2/TLR6 agonist) and PAM3CSK4 (TLR2/TLR1 agonist). TLR-4 activation results in the expression of key immunomodulatory cytokines and chemokines (e.g., IL8, IL6, CCL2, CXCL1) via the MYD88 dependant signalling pathway. Moreover, TLR-4 activation specifically induces activation of the Type 1 Interferon signalling pathway.

**Conclusions** The data shows that DPSCs have underappreciated immunomodulatory properties and play a role in the defence of dental pulp against microbial invasion. In addition, DPSCs may also play a role in inflammatory pain via a Type 1 interferon response. The findings reported therefore may allude to novel therapeutic targets to treat inflammatory or neuropathic pain.
Link Between Ageing and Autophagy in Human Pulpal Odontoblasts
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Objectives Aged post-mitotic cells accumulate biological waste as defective mitochondria and non-degradable protein aggregates. The mechanisms of ineffective waste disposal systems are not well understood in human pulp. The aim of the study is to examine and compare the immunohistochemical distribution of COX2, LAMP2 and MAP LCIi3 in human odontoblastic cells depending on age, gender, tooth type and cell topography (crown/root areas).

Methods Ninety intact teeth of healthy individuals were enrolled in the study and arranged in three groups (n=30 in each group) regarding the patients’ age: tooth germs with young pulp (14-16 years old), adult/mature pulp (18-40 years old) and senescent pulp (41-78 years old) groups. All teeth were freshly extracted due to orthodontic indications (dental germs of third molars), difficult eruption (third molars) and periodontal and bone lost (premolars and incisors). The specimens were fixed overnight in 10% buffered paraformaldehyde, decalcified in a 3% hydrochloric acid (HCl) for 6 hours and paraffin embedded. Immunohistochemistry using mouse monoclonal antibody COX2 (D-5), LAMP2 (H4B4), and MAP LCIi3 (G2) Santa Cruz Biotechnology Inc., USA, was performed by Leica-Bond Max automated system (Leica Biosystems, Germany). Statistical analysis including Kruskal-Wallis and Mann-Whitney tests were applied (p<0.05) by IBM SPSS Statistics 25.

Results In aged dental pulp, immunohistochemically odontoblasts expressed statistically significant more COX2 and MAP LCIi3 (p<0.05). A greater expression of LAMP2 was shown, but no statistically significant difference was identified (p>0.05). The expression of all biomarkers was not statistically different in upper/lower, posterior/frontal teeth, as well as in males/females specimens (p>0.05). Coronary odontoblasts expressed statistically significant more biomarkers than radicular cells (p<0.05).

Conclusions Insufficient recycling by mitophagy in aged odontoblasts shows accumulation of mitochondria with defective respiratory functions and further damage of degradation systems resulting in energy cell deficiency and over-oxidative stress. Possible link between ageing and autophagy confirms mitochondrial-lysosomal theory of ageing in odontoblasts.

Cell-Homing Versus Cell-Transplantation Approach for Dental-Pulp Regeneration: a Systematic Review
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Objectives The main objective of this systematic review was to compare the apical healing, root maturation and histological characteristics of teeth treated with the cell-based versus the cell-free techniques.

Methods A literature search strategy was carried out on PubMed, EMBASE and the Web of Science databases. The last search was done on 1 August 2021. No filters or language restrictions were initially applied. Two researchers independently selected the studies and extracted the data. As randomized clinical trials were not available, animal studies were included.

Results In total, 26 studies were included in the systematic review. In terms of apical healing, the qualitative analysis of the data suggested that there seems to be no significant difference between the cell-free and the cell-based techniques. The results regarding root maturation of immature teeth are contradictory. Two articles concluded that the cell-based technique results in significantly more apical closure than the cell-free technique whilst another article concluded that there was no significant significant difference between both techniques regarding root maturation. The main difference between the cell-free and the cell-based techniques seems to be the histology of the treated tooth. The cell-free technique seems to result in cementum-like, bone-like or periodontal ligament-like tissue. One study, on the other hand, found that the cell-based technique resulted in regeneration of the whole pulp with an odontoblast layer, connective tissue, blood vessels and neuronal tissue.

Conclusions Currently, the amount of randomized clinical trials on this topic are very scarce. This is probably due to the limited infrastructure and lack of resources to apply the cell-based technique. Even though both techniques seem to be promising for clinical application, long-term data needs to be provided regarding the healing and reparative patterns after regenerative endodontic therapy.
Immunohistochemical Study of Inflammatory and Angiogenic Response of Dental Pulp

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Objectives
This study was undertaken to examine the presence and quantitative changes of class II antigen-presenting cells, T and B lymphocytes, and endothelial cells in the dental pulp in healthy and carious human teeth.

Methods
In this study, we have examined 60 maxillary/mandibular premolars under 3 different clinical conditions: healthy teeth, and shallow and deep cavities. Teeth were extracted for various therapeutic reasons (mostly for orthodontic reasons), and immediately cut longitudinally; pulp tissue was extirpated and fixed in formalin for 24 hours at 4 °C. The specimens were embedded in paraffin, according to standardized laboratory procedures. Sections were cut at 5 μm thicknesses and stained by the streptavidin-biotin complex immunoperoxidase method. Cells were identified by using the following monoclonal antibodies: HLA-DR, CD68, CD45, CD20, and CD34.

Results
In the pulp of healthy teeth, HLA-DR-positive cells were distributed mainly under the odontoblast layer, with few CD68 positive cells located more coronary and with single CD45 and CD34 positive cells. Pulpal immunological response in shallow dentinal caries was characterized by a localized accumulation of HLA-DR antibody-positive cells. CD68 positive cells were present in a small number located coronary. The number of CD45 lymphocytes showed an increase in teeth with shallow dentinal caries. In the pulp of progressed caries, distinct quantitative and qualitative differences in inflammatory cell infiltration were observed with a marked increase of HLA-DR-positive, CD45, and CD20 positive cells. In carious teeth, CD34 positive cells subsequently coalesce to form solid vascular cords inside the connective tissue, which later aggregate with the progression of the carious lesion.

Conclusions
This study suggests that immune mechanisms play important role in pulpal infection resistance. Dental caries revealed distinct quantitative and qualitative differences in inflammatory cell infiltration. The presence of CD34 endothelial cells reveals the continuous adjustment of vessels in response to functional needs.

Debridement of Root Canal Isthmus by Laser Activated Photoacoustic Streaming

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Objectives
This study aimed to evaluate the efficacy of a new Er:YAG laser assisted irrigation system, Shock Wave Enhanced Emission Photo-acoustic Streaming (SWEEPS), in the removal of pulp tissue from root canal isthmus area in lower molars, and to compare it with ultrasonically activated irrigation (UAI) and conventional needle irrigation (CI).

Methods
The study sample consisted of 41 lower molars with the presence of an isthmus between mesial canals based on cone-beam computed tomographic analysis. The selected teeth were randomly distributed into experimental groups (n=12/each) and a control group (C) (n=5). The traditional access cavity of the mesial part of each tooth was made in all samples. In the experimental samples, the mesial root canals were instrumented with Wave One Gold Primary (25/.07) file using 3ml of 3% sodium hypochlorite (NaOCl), during 5min. The distal canal served as control of the presence of pulp tissue in the tooth. Then, the root canals were irrigated with final irrigation protocol (FIP) using one of the following techniques: SWEEPS; UAI; CI. The FIP included sequential application of 3ml 3% NaOCl (60s), 2ml ethylenediaminetetraacetic acid (EDTA) (60s) and 3ml 3% NaOCl (30s). In C, no treatment was performed. Sections from the isthmus region (3-4mm from the apex) were processed for histologic evaluation to measure remaining pulp tissue (RPT). The results were analysed using ANOVA and Kruskal-Wallis test (α=0.05).

Results
No significant differences in the relative surface area of root canals and isthmus was reported among groups (p>0.05). Samples in the SWEEPS group had significantly less remaining pulp tissue (ranging between 1.4-7.4%) than UAI, CI and C (p=0.003, 0.014, 0.003). There were no difference between the UAI and CI (p=0.583).

Conclusions
SWEEPS was the most efficient in debridement of root canal isthmus area, and UAI and CI showed similar but lower efficiency.
Should Separated-Files be Removed? a Stress-Analysis Study About Profit-Loss

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Objectives The aim of this finite element stress-analysis (FEA) study was to assess the effect of separated-files (SF) which were broken at two levels (at apical 4 and 9mm) on biomechanics of premolars and to determine if removal-techniques have an effect on stresses.

Methods Six lower premolar-teeth were screened on computerized microtomography (micro-CT), standard access-cavities were prepared, working-lengths were determined and root-canals were prepared (Protaper files, Protaper Universal, Dentsply, Switzerland). Notches were created on the final shaping-file F3 at a point 4 and 9mm from the file-tips, inserted in the canals and squeezed at the apical to break. The roots were re-screened and SFs were removed by using Masseran kit (MK; Micro Mega, France), ultrasonic-tips (UT; Satelec, Acteon group, France) and by-pass (BP) technique. Final scans were obtained and converted into three dimensional FEA models. The following conditions were then simulated: a) root-filled tooth with SF; b) root-filled tooth after removal of the SF by using with MK, UT or BP techniques. The models were loaded axially (from two directions) and vertically (300 Newton), Von mises stresses were then analyzed.

Results SFs increased the stresses within the root. Longer SF caused more stresses within the root thus should be removed while short SFs, if cannot be removed could be kept considering the other clinical factors. Among the techniques used, removing with MK caused more stresses within the roots. Axial loading caused more stresses at cervical while the stresses moved towards the apical under vertical loading.

Conclusions SFs increase the stresses within the root. Long SFs should be removed while short SFs can be left (considering other clinical factors that may affect the success of the treatment). Trying to remove short SFs located at apical negatively affects biomechanics of the teeth and this effect is more dramatic with MK technique.

Mechanisms and Loss Rates of Enamel Using air-Abrasion and Nanoimpact

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Objectives To use air-abrasion to determine whether engineering erosion theory can be applied to enamel to differentiate the damage mechanisms (brittle or ductile) using rates of loss at different angles and resultant scar shape. In addition, nano-impact will be used to determine if the link between D/d ratio and damage mechanism, (D: contact footprint diameter of the impacting particle, d: column diameter) for columnar structured engineering materials also applies to enamel.

Methods Bovine enamel samples (n = 10) were subjected to air abrasion for 5 periods of 2 seconds, at an air pressure of 5 Bar using a 54 µm Al2O3 powder. The incident angle of the particle flow was set at either 90° (n = 5) or 45° (n = 5). The samples were weighed (scales accurate to 0.01 mg), and the scar imaged after each period using a Proscan 2100 (Scantron, Taunton, UK). A bovine enamel samples was subjected to 10 nanoimpacts at a load of 10mN at flight distances of 4, 8, 12, 16, 20 and 24µm for each of the spherical tip sizes: 5, 10, 50, 100, 200, 500 µm. The coefficient of restitution (COR), dynamic hardness (DH) and dynamic depth (DD) was determined for each impact.

Results The air-abrasion testing demonstrated a higher loss rate when particle impingement was at 90°, with u shaped scars, indicating a brittle loss mechanism. The nanoimpact experiments showed a direct linkage between the nanoimpact response and the D/d ratio, with a clear inflection in the COR and DD when the D/d ratio was <2.

Conclusions The air-abrasion of enamel enabled the application of engineering erosion theory to identify the brittle behaviour of enamel during air abrasion, while the nanoimpact experiments demonstrated the influence of the D/d ratio on the enamel’s response to single particle impacts.
Sterility Level Over Time of Multi-Used Sterilization Pouches
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Objectives The issue of sterilization in dental clinics has a crucial impact on the dental clinic’s daily routine. On one hand there is a demand for a high standards of infection control, and on the other hand a need for high tool availability. To achieve the realization of these needs, the common practice is using sterilization bags (or pouches). One of the major issues in management of any dental practice nowadays is waste management and the environmental sustainability impact of the practice. To reduce environmental harmful impacts, some clinics reuse sterilization pouches several times despite most manufacturers suggestions for a single usage. The objective of this study was to test the sterility level of items packed in reused pouches, over time.

Methods 120 sterilization bags were prepared, with a nickel-titanium endodontic file inside, as the tested item. The bags were divided into groups according to the sealing method, and number of prior sterilization cycles. After standard sterilization, we stored them for four weeks, during which every week one group of bags was opened, and a biologic test was performed to assess bacterial colonies development, according to a recognized protocol of incubation and sowing on a BHI substrate.

Results Only three bags out of 120 developed an infection (and these were only after four weeks –and two of them from the control group, the non-sealed ones)

Conclusions The findings of this study indicate that there is a good basis for reuse of sterilization bags, at least in terms of maintaining the level of sterilization. Additional research is required with conditions which are more similar to reality, as well as to examine the practical sides of reusing sterilization bags. As environment awareness in the world increases, these findings are important.

Effects of Different Whitening Agents on Resin Composites’ Esthetic Properties
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Objectives The aim of this study was to examine the effects of different whitening agents on the color and translucency of different resin composites, in vitro.

Methods A total of 315 specimens (10.0×2.0 mm) were fabricated from two microhybrid (G-aenial anterior[G-Ant]) and (G-aenial posterior[G-Post]) and a nano hybrid (G-aenial A’CHORD[G-ACH]) resin composites and each group was randomly distributed into seven experimental groups (n=15) as follows; 1- control (C); 2- in-office whitening agent (40%Hydrogen Peroxide[HP], Opalescence Boost, Ultradent, South Jordan, USA[IOW]); 3- at-home whitening agent (Opalescence,10% Carbamide Peroxide [CP], Ultradent, South Jordan, USA[AHW]); 4- prefilled tray (Opalescence Go, 6%HP Ultradent, South Jordan, USA[PT]); 5- whitening pen (6% HP, Cavex Bite&White, Cavex, Haarlem, Nederland[WP]); 6- whitening toothpaste (Opalescence, Ultradent, South Jordan, USA[WT]) and 7- whitening mouth rinse (2% HP, Listerine Advanced White, Johnson & Johnson, New Jersey, USA[WMR]). The composite specimens were subjected to staining except control group and then to whitening. The color of specimens was measured after 24 h (T0), after staining (T1) and after whitening (T2) and changes in whiteness index (WI), CIEDE2000 (ΔE00) and translucency parameter (TP) values were calculated. Data were analyzed statistically (p<0.05).

Results No significant WI differences were detected among the tested resin composites at T0 and T1 (p>0.05) whereas a significant difference was seen at T2 (p<0.005). AHW and IOW produced higher color change than PT, WP, WT and WMR. Significant TP changes were seen after the application of IOW and AHW in G-Ant.

Conclusions The effect of whitening products on the whitening index, color and translucency of resin composites are material and substrate dependent. Clinicians should know that existing resin composite restorations are prone to discoloration and effectiveness of the whitening agent used for color recovery of discolored restorations varies depending to the type of the material. Thus, replacement may be required.
Effect of Bleaching on Microhardness and Color-Change of Resin-Infiltrated Lesions
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Objectives The aim of this study was to evaluate the effect of resin infiltration and bleaching treatments on microhardness and color change of initial enamel caries lesions.

Methods Thirty-nine human incisors were used in this study. The teeth were divided into 3 groups (n=13): Group-A: Sound-teeth control group; Group-B: Artificially-induced enamel caries; Group-C: Resin infiltrated artificial enamel caries.

Microhardness values of the specimens were determined by HMV Microhardness Tester (Shimadzu, Japan)

VHM: Initial microhardness measurement (applied to Group A, B and C)
VHMd: Microhardness measurement after demineralization regimen for artificially-induced enamel caries (applied to Group B and C)
VHMr: Microhardness measurement after resin infiltration (applied to Group C)
VHMss: Microhardness measurements after staining solution (coffee, 1 week) (applied to Group A, B and C)
VHMb: Microhardness measurements after bleaching treatment [18% hydrogen-peroxide gel (Biowhiten, Prooffice)] (applied to Group A, B and C).

The color changes of the teeth in all groups were evaluated after coffee staining and bleaching using spectrophotometer (Vita Easy Shade Advance4.0, VITA Zahnfabrik, Germany). Measured CIE L*, a*, and b* at each point were compared and color difference (ΔE) was calculated.

The statistical comparison between groups was performed using the Variance analysis for repeated measurements, Bonferroni test was used for binary comparisons (p=0.05)

Results After bleaching, similar VHMb values were detected between Group A and C (p>0.05). VHMb showed significantly lower microhardness value than VHMss in Group B (p<0.05). VHMb showed significantly higher microhardness value than VHMss in Group C (p<0.05).

ΔE2 value is significantly lower than ΔE1 value in both Group A and Group C (p<0.05) There was no difference between ΔE2 values of Group A, B and C (p>0.05)

Conclusions After bleaching, color change of stained sound teeth was similar with the color change of stained enamel caries lesions with and without resin infiltration.

After bleaching, the resin infiltrated group showed higher microhardness values than the non-infiltrated group, while this value was similar to the sound-teeth control group.

Heating Effect Upon the Color Coordinates of Composite Resins
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Objectives To evaluate the color changes of 3 composite resins (2 universal and 1 microhybrid) after one cycle of heating.

Methods A total of 84 disks (n=14) (diameter 10 mm, thickness 1 mm) were manufactured from 2 universal composites (Omnichroma- Tokuyama and Optishade universal MD – KavoKerr) and 1 microhybrid composite, (Biofunction Enamel Plus Hri, Micerium). From the total number, half of the specimens were made from unheated composite, the other half were fabricated after the composites were subjected to heating (45 °C, 1h, Ease-it, Ronvig).

The specimens were incrementally built (Porcelain sampler, SmileLine), polymerized on both sides (Woodpecker Led. H Orto), and immersed (24 h) in distilled water.

The color coordinates (L*, a*, b*) have been measured with a spectrophotometer (SpectroShade (MHT), with the samples placed on white, black, and grey background.

Differences in color parameters ΔL*, Δa*, Δb*, color and translucency difference (DE00 and ΔTP00), and difference in whiteness index (ΔWID) between unheated and heated samples have been calculated.

Results The calculated differences varied as follows: ΔL* -0.35 – 0.98; Δa* -0.27 – 0.0; Δb* -2.18 – 0.06; DE00 0.79 – 1.76; ΔTP -2.03 - -0.18, and ΔWid 0.02 – 3.54. In all cases, Omnichroma had the highest variations of the optical parameters.

Conclusions One cycle of composite heating resulted in color and translucency differences; for the universal composite DE00 was situated between the perceptibility and acceptability thresholds.
Can We Repolish Discolored Single-Shaded Composite Resins With Different Wheel-Polishers?
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Objectives To evaluate the color restitution of single-shaded composite resins immersed in a coffee solution and repolished with different wheel systems.

Methods Twenty-four-disc samples of 8x2mm (n=120) were prepared in plastic molds for Omnichroma (OMN; Tokuyama, Japan), Zenchroma (ZEN; President, Germany), Essentia Universal (EU; GC, Japan), Charisma Diamond One (CDO; Kulzer, Germany) and NeoSpectra (NS; Dentsply, Germany). One side of the samples was left unpolished (Mylar strip) while the others were randomly polished with Twist-Dia (TWD) or Nova-Twist (NOV) for 30 sec. Specimens were immersed in a coffee solution for seven days. Color measurements according to CIEL*a*b* system were performed with a spectrophotometer (Vita Easyshade, Vident, Brea, USA) at baseline, after seven days, and after repolishing. The color change (ΔE*) values were evaluated by T-test, Multivariate ANOVA, and Dunnett-t post-hoc tests (p<0.05).

Results The coffee discolorations that occurred were above the clinically acceptable level of ΔE = 2 in all groups. Regardless of the materials and the polishing systems used, Mylar strip groups were the more discolored ones. In TWD groups, CDO was the most discolored group (ΔE=6.45) while OMN (ΔE=2.96) and ZEN (ΔE=3.06) were the less (p=0.000). In NOV polished materials, EU (ΔE=5.85) and CDO (ΔE=5.68) were similarly the most discolored groups (p=0.05) while NS, OMN, and ZEN were the less (respectively 3.48; 3.70; 3.79). After repolishing, regardless of the polishing systems used, CDO could not reach the level of ΔE=2 (p<0.05) while the other groups had values far below. The type of polishing system used affected the ΔE values after storage in coffee for CDO (p=0.015), OMN (p=0.000), and ZEN (p=0.011). Compared to TWD groups, NOV groups were less discolored in CDO and NS while more in EU, OMN, and ZEN.

Conclusions Color changes after coffee immersion were perceptible in all single-shaded composite resins but repolishing could restitute the color at a level of less than ΔE=2.

Surgical Results of Immature Third Molar Autotransplantation Using Printed Replicas.
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Objectives The aim of the study was to compare surgical results of immature third molar autotransplantation without and with use of 3-dimensional printed donor tooth replicas. The replica was expected to decrease the total surgery time and the number of attempts of fitting the donor tooth to the recipient site.

Methods All surgeries were done in RSU SI. Planning and replica printing for surgeries were supported by BBCE. The replica was created based on the cone-beam computed tomography data of the patient and subsequently 3-dimensional printed. Two groups of immature third molar autotransplantation were formed, one conventional (group A) and one using a printed donor tooth replicas (group B). Total surgery time from the first incision to the last suture, donor tooth extra-alveolar time and the number of donor tooth fitting attempts were monitored.

Results Group A consisted of 22 patients, (6 males, 18 females, mean age 17.81, range 13-22). The average total surgery time was 65.91 minutes, donor tooth extra-alveolar time was 63.63 seconds, and the average number of fitting attempts was 2.00.
Group B consisted of 19 patients, (8 males, 11 females, mean age 16.10, range 14-19). The average total surgery time was 45.00 minutes, donor tooth extra-alveolar time was 61.32 seconds, and the average number of fitting attempts was 1.53.

Conclusions The use of a replica made the procedure less traumatic, reduced average total surgery time by 20.91 minutes, the donor extra-alveolar time by 2.31 seconds, and the number of fitting attempts of the donor tooth by 0.47.

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O082

CBCT-Based Biomechanical Models for the Study of Tooth Auto-Transplantation Outcomes

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Objectives This study aimed to use CBCT-based biomechanical models for the analysis of tooth auto-transplantation outcomes.

Methods 3D models were created based on CBCT scans of 6 patients (mean age 11.54 y ± 0.81) having undergone tooth auto-transplantation from the premolar region to the anterior-maxillary area (EC: S55287). Included patients had a pre-operative and a one-year follow-up CBCT. All scans were registered and segmented into their different structures; bony structures (with cortical, trabecular and sclerotic bone), teeth (including enamel, dentin and pulp tissue), and a simulated periodontal ligament. Contralateral central incisors were analysed as negative controls.

Models were then meshed using Desk Software, and finite element analyses were performed based on a design-of-experiment approach. All dental materials were modelled to be homogeneous and linearly elastic except for the periodontal ligament, which was modelled hyper-elastic. The attributed material properties were referenced from the literature. A perfect bonding between each component was simulated, and an oblique load of 300 N was applied to simulate masticatory forces. The nodes of the lateral faces of the cortical bone were constrained to prevent displacement. The finite element analysis was conducted on the software Abaqus (Dassault Systèmes, Vélizy-Villacoublay, France) to calculate the von Mises stresses occurring at the level of the tooth and surrounding bony structures.

Linear and volumetric measurements were finally performed to assess tissue change at the root level at different time points.

Results Preliminary results suggest statically significant differences in the distribution of von Mises stresses between all six cases of auto-transplanted teeth. In contrast, no statistically significant differences between control central incisors were noted between the different time points.

Conclusions Accurate patient-specific modelling (PSM) combined with finite element analyses could help us understand the influence of stress distribution on root formation following TAT and could further open doors regarding our understanding of oral biomechanics and their impact on tooth root tissue remodeling following TAT.
DFDBA Allograft to Accelerate the Healing in Lefort’s Osteotomy Sites

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Objectives: to evaluate the effects of demineralized freeze dried bone allograft (DFDBA) with putty consistency on the healing process of Lefort’s osteotomy sites in orthognathic surgery.

Methods: This split-mouth study was performed on patients requiring Lefort’s osteotomy for surgical advancement of the maxilla after orthodontic treatment. After the surgical procedures and before soft tissue suturing, the osteotomy site on one side of the maxilla was randomly selected for placement of DFDBA, while the other side was considered as the control site. Computed tomography (CT) scans of the patients were obtained preoperatively and 4-month postoperatively. The area of the osteotomy defects in three-dimensional frontal and lateral reformations were compared before and after the surgical procedure on both sides. The data was then statistically analyzed (α=0.05).

Results: 20 patients participated in this study. Preoperatively, the area of the defects on the control and DFDBA sites were not significantly different (P=0.810). After 4 months, the area of the defect was not significantly different between DFDBA and control sites (P=0.628).

Conclusions: Application of DFDBA with putty consistency is not effective on healing of Lefort’s osteotomy sites. Therefore, it is not recommended for routine use in orthognathic surgeries for the purpose of healing acceleration.

Refined rat Model for Mandibular Osteoradionecrosis Study and Treatment

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Objectives: Radiation therapy is widely used for head and neck cancer treatment. Despite its advantages, it can be responsible for many orofacial complications. OsteoradioNecrosis (ORN), characterized by necrotic and exposed bone tissue without healing over a 3-month period, is considered the most devastating complication. Most management strategies of ORN involve surgical intervention, and recurrence is common, hence the need for new treatments. However, the lack of knowledge about ORN pathophysiology limits their development. In this study, we developed a rat mandibular ORN model to study bone and vascular changes, for better understanding of ORN pathogenesis, and to investigate the efficacy of a new potential treatment.

Methods: To define the ORN model, we tested the impact of radiation doses of 20 to 40 Gy (medical linear accelerator delivering 4-10 MV X-rays), combined with dental extraction, on the mandibles of Lewis rats during a 4-month period. Micro-computed tomography (µCT, Quantum GX2, Perkin Elmer) imaging system was used to obtain in vivo images for longitudinal tracking of bone volume and ex vivo images, after animal perfusion with barium sulphate contrast agent, to track the volume of the vascular network.

Results: We defined 25 Gy as the minimum dose of irradiation combined with dental extraction required to develop non-regenerative bone necrosis. Quantitative µCT findings were correlated with clinical and histological analyses. The decrease in bone volume in irradiated rats from the first weeks was associated with an early decrease in vascular volume compared with controls, suggesting a role of ischemia on the biological manifestations of ORN. We also had preliminary data on the implementation of a local treatment with minimally-invasive surgery, based on an original biomaterial cell-assisted therapy procedure.

Conclusions: This refined model allowed us to study the development of ORN, which helps to better understand this pathology and to test the effectiveness of a new biomaterial cell-assisted therapy.
**Odontogenic and Non-Odontogenic Maxillofacial Infections During COVID-19 Lockdown: Retrospective Analysis**

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**Objectives** To evaluate the impact and effect of COVID-19 lockdown on the epidemiology of maxillofacial odontogenic and non-odontogenic infections at Vilnius University Hospital Zalgiris Clinic.

**Methods** Retrospective data analysis of patients, presented with odontogenic and non-odontogenic oral and maxillofacial infections during COVID-19 lockdown in 2020 from 03.17-04.17 was performed and compared with the same period in 2019. The followong data were collected from the patients medical records: type and cause of infection, duration of symptoms prior to hospital admission, treatment provided, onset of disease. Data analysis was performed using IBM SPSS software, Mann Whitney U test, Student’s t test, Pearson’s Chi Square test were used to analyse data, the threshold for statistical significance for all tests was set at p<0.05.

**Results** There was a total of 1000 patients included in the analysis (n=703 in 2019 and n=297 patients in 2020).

The frequency of maxillofacial infections have significantly decreased during a lockdown period (p<0.05). Significantly less patients were hospitalized during lockdown period (p>0.05), but the hospital stay was significantly longer (2.8 days 2019, 4.7 days 2020, p<0.05). The most common cause of infection was periapical pathology, as a complication of untreated caries lesion (78.8%, n=788). The waiting time after the onset of symptoms was significantly longer during lockdown (4 days compared to 2.4 days, p<0.05). In 2020, significantly more patients required advanced surgical treatment (2019 - n=244 (34.7%); 2020 - n=126 (42.4%), p<0.05).

**Conclusions** A national lockdown caused a considerable decrease of patients presenting with odontogenic and non-odontogenic oral and maxillofacial infections. Delays in referral due to lockdown resulted in more severe infections requiring extensive surgical treatment and longer hospital stay.

**Evaluation of Three Different Suture Materials With Thermal Aging Technique**

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**Objectives** In the secondary intention of wounds, the suture should maintain its strength to bring the two wound edges side by side until the healing phase is over. We evaluated the strength of three different suture materials, kept in artificial(AS) or human saliva(HS). In addition, the thermal aging technique was applied for real-like outcomes.

**Methods** Poly(glycolide-co-lactide)(PGLA) and poly(glycolide-co-caprolactone)(PGCL) were used as absorbable and silk was used as a non-absorbable suture. The sutures kept HS or AS according to the group they belong to. All suture materials aged with thermal cycle. Tensile strengths were tested at the initial, day 3, day 7 and day 14.

**Results** The mean tensile strength of all suture types was statistically different in the initial testing(p<0.01). In the silk type, only Day3-AS was not statistically different from the initial test(IT). In the PGLA type, statistical differences were found in all tests between IT. In type PGCL, there were statistically differences in all measurements between IT, but Day3-AS and Day3-HS. In the evaluation performed on the third, seventh and fourteenth days, the difference between artificial and human saliva was found only on the 14th day in the PGCL type.

**Conclusions** There is no statistical difference between HS and AS for seven days, the use of AS may give accurate results in studies that will last less than seven days. In longer studies, it would be more appropriate to use HS to ensure accurate results.
Comparison of 1-Stage and 2-Stage Palatoplasty: a Systematic Review and Meta-Analysis
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Objectives The choice between different palatoplasty procedures for a complete cleft lip and palate repair is a challenge in maxillofacial surgery worldwide. There is an unmet medical need to provide guidance for this question. Therefore we aimed to evaluate and compare the maxillofacial and speech development after 1-stage and 2-stage palatoplasty.

Methods This meta-analysis was performed using Medline, Embase and Cochrane Central database until March 2022 with the PROSPERO registration number CRD42021289846. Our methodology is based on the PRISMA statement. All the studies were retrospective and cross-sectional studies written in English. The analyzed outcomes included lateral cephalometry (SNA, ANB, SN-PP, U1-PP), audible nasal air leakage, velopharyngeal insufficiency, hypernasality and anterior cross-bite. The results were analyzed using the Random effect model, represented as standardized mean difference (SMD) and Odds ratio (OR). The Risk of Bias was assessed by the ROBINS-I tool, while the level of evidence by GRADE analysis.

Results From a total of 194 articles after our systematic search 17 studies with 885 patients were eligible for analysis. A statistically significant difference was found for the U1-PP, audible nasal air leakage and velopharyngeal insufficiency outcome, favoring the 1-stage approach (SMD 2.35 [0.18; 4.15]; OR 0.27 [0.07; 1.00]; OR 0.27 [0.08; 0.85], respectively). The statistical analysis of hypernasality showed a tendency toward lower odds, favoring the 1-stage method (OR 0.49 [0.17; 1.40]). For SNA, ANB, SN-PP and anterior cross-bite no superiority could be shown favoring one method over the other (SMD -0.28 [-1.32; 0.75]; SMD -0.4 [-1.26; 0.45]; SMD -0.31 [-1.59; 0.97]; SMD 1.40 [0.82; 2.41] respectively).

Conclusions 1-stage palatoplasty provides better speech development, while maxillofacial development does not differ substantially between 1-stage and 2-stage surgery. Thus the 1-stage method may serve as the first choice for operation unless obstacles appear in individual patients.

The Effect of Consent Form on Anxiety in Oral Surgery
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Objectives In our study, it was aimed to investigate the psychological and physiological effects of the medical information in the consent forms prepared for the impacted third molar tooth extraction on the patients.

Methods This study was carried out on a total of 100 patients, 50 women and 50 men. The patients do not have any psychological or physiological diseases. The patients were selected from those who had not had impacted third molars removed before. The Visual Analogue Scale (VAS) and Modified Dental Anxiety Scale (MDAS) were used to determine the anxiety scores of the patients; blood pressure, oxygen saturation and pulse values were measured to determine vital values.

All patients, before and after reading the consent forms; MDAS and VAS scales were directed twice and vital signs of the patients were measured. According to the answers given by the patients to the scales and their vital signs, the effect of the information in the consent forms on the patient’s anxiety was evaluated.

Results As a result, when informed consent is given adequately and clearly; It is seen that the anxiety scores of the patients decreased significantly and their vital signs became stable.

Conclusions The patient’s reading of the detailed consent form before the operation provides a safer environment for both the patient and the doctor.
Mucoadhesive Patches Containing Antibody Derivatives Reduce Inflammation in Oral Lesions
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Objectives Chronic ulcerative oral mucosal inflammatory diseases, including oral lichen planus (OLP) and recurrent aphthous stomatitis (RAS), are painful and highly prevalent, yet lack effective clinical management. The pro-inflammatory cytokine TNFα is a key molecule in the pathogenesis of these diseases. The ability to deliver TNFα-neutralising biologics topically to the oral mucosa would greatly expand treatment options. This study aims to evaluate a mucoadhesive patch formulation for the delivery of anti-TNFα-F(ab) antibody fragments to the oral mucosa.

Methods Mucoadhesive fibrous polymer patches containing F(ab) antibody fragments were prepared using electrospinning and characterised. Patches containing neutralising anti-TNFα F(ab) were applied to tissue-engineered oral ulcer models containing activated macrophages to measure the effect on pro-inflammatory cytokine concentrations.

Results The polymers used in the formulation had a protective effect on F(ab) functionality and facilitated patch fabrication by electrospinning. The F(ab) were rapidly released from the patch in aqueous media (97 ± 5% released within 3h). Neutralising anti-TNF-α F(ab) fragments were generated by papain cleavage and incorporated into patches. Patches containing anti-TNFα F(ab) were found to have TNF-α neutralising activity, as shown by the suppression of TNF-α-mediated CXCL8 release from oral keratinocyte grown as monolayer cultures. Anti-TNFα patch treatment led to reduced levels of active TNFα along with a reduction in the levels of disease-implicated T-cell chemokines (CCL3, CCL5, and CCL10) to baseline concentrations.

Conclusions Topical antibody delivery using this formulation has the potential to change the way debilitating oral diseases such as OLP and RAS are treated in the future, as well as representing a platform technology for the site-specific delivery of antibody fragments to tissue surfaces to treat a wide range of conditions.

Effects of Children’s Toothpastes and Detergent Contents on Zebrafish Embryos
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Objectives We aimed to evaluate how toothpastes for children affected molecular mechanisms of odontogenesis in zebrafish embryos. Toothpastes (TP) were selected according to their detergent contents, with cocoamidopropyl betaine (CAPB) (Sensodyne Promine 6-12 age, TP1) and sodium lauryl sulfate (SLS) (Colgate +6, TP2). TP3 contained no detergent (Negative control, Jack n’ Jill +6). Effects of SLS and CAPB alone were also examined.

Methods Toothpastes and detergent concentrations affecting development were determined as 750 mg/L and 4 mg/L respectively. Embryos were exposed to TP1, TP2, TP3, SLS, CAPB and embryo medium (Control) for 72 hours post-fertilization. Acetylcholinesterase (AChE) activity and oxidant-antioxidant parameters were analyzed spectrophotometrically. Expressions of tooth development genes were evaluated by RT-PCR. Intraocular distance, lower jaw and ceratohyal cartilage length were displayed using alcian-blue staining.

Results axin2 and wnt10a expressions increased in SLS and TP2 groups. igf2a and eve1 expressions decreased in all groups except TP3. nrOb1 expression decreased in TP1, SLS and CAPB groups. Oxidant-antioxidant balance was disturbed in all groups except TP3, evidenced by increased lipid peroxidation and nitric oxide. SLS and CAPB groups were more affected in terms of AChE, glutathione S-transferase and superoxide dismutase; perturbations were observed in cartilage structures.

Conclusions Axin2 functions in Wnt signaling pathway activating tooth formation. Altered expression of tooth development gene axin2 correlated with wnt10a, and with changes in cartilage structures in SLS and TP2 groups. TP3 group presented no disruptions in oxidant-antioxidant balance. Our study shows the availability of externally-developing zebrafish embryos in examining the effects of toothpastes’ contents on embryogenesis.
White Matter Hyperintensities in Burning Mouth Syndrome

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Objectives
White matter hyperintensities (WMHs) of the brain are observed in normal ageing, in various subtypes of dementia and in chronic pain, playing a crucial role in pain processing. The aim of the study has been to assess the WMHs in Burning Mouth Syndrome (BMS) patients by means of the Age-Related White Matter Changes scale (ARWMCs) and to analyze their predictors.

Methods
100 BMS patients were prospectively recruited and underwent Magnetic Resonance Imaging (MRI) of the brain. Their ARWMCs scores were compared with those of an equal number of healthy subjects matched for age and sex. Intensity and quality of pain, psychological profile, and blood biomarkers of BMS patients were further investigated to find potential predictors of WMHs. Specifically, the Numeric Rating scale (NRS), Short-Form McGill Pain Questionnaire (SF-MPQ), Hamilton rating scale for Depression and Anxiety (HAM-D and HAM-A), Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS) were administered.

Results
The BMS patients presented statistically significant higher scores on the ARWMCs compared to the controls, especially in the right frontal, left frontal, right parietal-occipital, left parietal-occipital, right temporal and left temporal lobes (p-values: <0.001, <0.001, 0.005, 0.002, 0.009, 0.002 and < 0.001, respectively). Age, a lower educational level, unemployment, essential hypertension, and hypercholesterolemia were correlated to a higher total score on the ARWMCs (p-values: <0.001, 0.016, 0.001 and 0.039, respectively). No correlation was found with the blood biomarkers, NRS, SF-MPQ, HAM-A, HAM-D, PSQI and ESS.

Conclusions
Patients with BMS showed a higher frequency of WMHs of the brain as suggested by the higher ARWMCs scores compared with the normal ageing of the healthy subjects. These findings could have a role in the pathophysiology of the disease and potentially affect and enhance pain perception.

Axial T2-weighted fluid-attenuated inversion recovery (FLAIR) images show multiple hyperintense signal of gliotic foci in the temporal (A), parietal (B) and frontal (C) white matter in a 60 year-old male patient affected by burning mouth syndrome

- Boxplot of systemic comorbidity and drug intake in BMS patients
- Boxplot of ARWMC total score of BMS and Control subjects according to the age subgroups

Median values are highlighted by bold lines.
Poor Oral Hygiene and Infective Endocarditis: a Case Control Study

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Objectives Infective endocarditis (IE) is an uncommon infection of the heart, particularly the heart valves, yet it is a condition associated with high morbidity and mortality, and one for which there is a lack of preventive strategies. Most IE prevention guidelines recommend maintaining good oral hygiene for IE prevention, but currently, no data exist that directly support this recommendation. The purpose of this case-control study was to determine if poor oral hygiene is associated with the subsequent development of IE among those considered at moderate risk for IE.

Methods Dental calculus (primary outcome measure), and other measures of oral hygiene and periodontal disease, were assessed among patients at moderate risk for IE who were recently hospitalized with IE (cases) and compared with matched controls, who were also at moderate IE risk, but had not developed IE. Blood cultures were obtained for all IE cases.

Results Case patients (n=62) had 53% greater mean dental calculus (difference = 0.29, 95% CI: 0.11, 0.48; p=0.002) and 26% greater mean dental plaque (difference = 0.18, 95% CI: 0.01, 0.36; p=0.043) than controls (n=119). Cases had fewer dentist and dental hygiene visits than controls (p=0.013) and fewer dental visits in the 12 weeks prior to enrollment (p=0.023). There were no significant differences between cases and controls in self-reported frequency of toothbrushing, flossing, or gingival bleeding on toothbrushing. Common oral bacteria were identified from blood cultures in 27/62 (44%) IE cases.

Conclusions Poorer oral hygiene, as reflected by increased calculus and plaque scores, and less frequent dental and dental hygiene care, was associated with the subsequent development of IE in this study. These results strengthen the rationale for recommending good oral hygiene as a means of reducing the risk of developing IE in all individuals considered to be at increased IE risk.
**O093 Oral Fibroblast’s Critical Role in Prevention and Treatment of MRONJ**

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**Objectives** Medication-related osteonecrosis of the jaw (MRONJ) is a severe complication that follows tooth extraction in oncologic patients receiving Zoledronic acid (ZA). While the mechanism in which ZA influences Osteoclasts is well known, the influence of ZA on Osteoblasts (OBs) differentiation and on oral mucosa hasn't been investigated thoroughly. Our aim was to investigate the interaction between soft tissue cells (STCs) and OBs after ZA administration.

**Methods** XTT proliferation assay was used to determine the ZA working concentration. STCs were represented by primary oral fibroblasts from three mucosal sites - PDL, masticatory and lining mucosa, and keratinocytes by HaCat cells. The conditioned media (CM) from all STCs was collected and served as secretome: CMFibro and CMHaCat.

Bone Marrow and PDL mesenchymal stem cells (MSCs) were cultured in osteogenic medium (OS). 5 μM ZA solution was added with and without STCs secretome and differentiation was evaluated using Alkaline Phosphatase, Alizarine Red and Von Kossa staining. Proteomic analysis was used to further investigate the difference in the content between CMFibro and CMHaCat.

**Results** Compared to MSCs cultured in OS, adding STCs secretome had no influence on OBs differentiation. However, addition of fresh 5 μM ZA solution with and without CMHaCat decreased OBs differentiation by at least 50%. Addition of CMFibro obtained from all fibroblasts, with fresh 5 μM ZA solution, enabled almost the same differentiation as in OS group. Proteomic analysis revealed the exclusive presence of specific proteins in CMFibro that participate in OBs differentiation.

**Conclusions** ZA decreased OBs differentiation and this effect was shown to be reversed by the addition of CMFibro. A possible mechanism of action may be the secretion of pro-osteogenic proteins by oral fibroblasts. In the future, this finding can be translated into clinical procedures, using connective tissue graft to seal an extraction socket in patients receiving ZA.

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**O094 Quantitative Histological Feature Analysis in Oral Epithelial Dysplasia**

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**Objectives** Oral epithelial dysplasia (OED) is the precursor to oral squamous cell carcinoma which is amongst the top ten leading cancers worldwide. Histopathological analysis remains the diagnostic gold standard; however, OED grading remains highly subjective and is an unreliable predictor of cancer risk. There is an increasing number of cytological and architectural abnormalities found in OED, although many features have not been quantitatively analysed or correlated to clinical outcomes. This study aims to quantitatively analyse differences in cellularity, nuclear and cytoplasmic features, and lesion thickness/perimeter in OED and evaluate the association of these features with malignant transformation and OED recurrence.

**Methods** Digitised whole-slide images of 75 OED and 25 control cases were analysed to quantitatively assess a range of histological features including: cell number, nucleus circularity, nucleus eccentricity, nucleus haematoxylin optical density (OD), cytoplasm eosin OD, nuclear to cell area ratio and lesion thickness/perimeter. Statistical analyses were conducted to analyse feature differences between OED grades, and to determine which features were predictive of malignant transformation and OED recurrence.

**Results** Findings showed an increased cell number in OED epithelium compared to control (p=0.039), and grade-related differences for cytoplasm eosin content (p=0.035), nucleus eccentricity (p=0.016) and nucleus circularity in basal epithelial cells of OED (p=0.0005). Nucleus circularity in OED epithelium was associated with lesion recurrence (p=0.018). Keratin perimeter was significantly increased in OED compared to control (p<0.00003) and epithelium perimeter in OED was strongly associated with malignant transformation (p=0.016).

**Conclusions** This study has revealed novel and potentially important differences in cellularity, geometric, colour and perimeter features in OED (p<0.05). Further validation on larger independent datasets is needed to establish the significance of these features in relation to OED progression.
Intraductal Irrigations and Sialoendoscopies Dual Treatment for Oral Dryness Relief

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Objectives To evaluate the combination of salivary gland intra-ductal irrigations (IG) followed by sialoendoscopy irrigations (SI) of the parotid gland on the improvement of salivary gland secretory dysfunction (SGSD).

Methods The study included a retrospective analysis of the medical files of patients with SGSD who were treated by dual therapy of major salivary gland IG followed by SI during 2014–2020. Collected data included the following parameters: socio-demographics, systemic co-morbidities, signs, and symptoms. Improvement was assessed by comparing the mean unstimulated and stimulated whole salivary flow rate (UWSF, SWSF) from the baseline point (before IG procedure) to the last point (after SI) using repeated measures. The between-subjects effects of various factors and covariants were analyzed using repeated-measures ANCOVA.

Results 100 patients were included with an age range of 15–83 years (mean age of 60.1±13.1 years). Improvement was detected on UWSF measurements (p=0.031, F=3.83), but not on SWSF measurements (p=0.165, F=1.85). The between-subjects effects on UWSF measurements were statistically significant for sex (p=0.003, F=9.526) and salivary gland manipulators use (p<0.001, F=15.107), and for the interaction between sex and salivary gland manipulators use (p=0.002, F=9.709).

Conclusions The combination of IG followed by SI increases UWSF salivary secretion in SGSD patients, and therefore can be used as a novel treatment modality to treat oral dryness in SGSD cases resistant to treatment.

Multidimensional Pain Evaluation Correlated With Psychological Profile and QoL in BMS

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Objectives The symptomatology in Burning Mouth Syndrome (BMS) is complex and it should be considered in accordance with a biopsychosocial model. The aim of this study is to evaluate the multidimensional aspects of pain with a complete battery of tests and to analyse its relationship with potential predictors such as mood disorders, sleep and quality of life.

Methods 40 patients with BMS vs an equal number of age and sex-matched healthy controls were enrolled. The VAS, SF-MPQ, BPI, PD-Q, BDI-II, STAI, PSQI, ESS, SF-36 and OHIP-14 were administered. The scores of the VAS, SF-MPQ, BPI, PD-Q, BDI-II, STAI, PSQI, SF-36 and OHIP-14 were statistically significantly higher in the BMS patients than the controls (p < 0.001**). A strongly linear correlation between severity and interference, while the contributions of the STAI and sleep were found to be statistically significant with the SF-MPQ and BPI in terms of severity and interference, respectively. Therefore, complete analysis of the patient requires several tools to better understand the multidimensional aspects of pain in BMS.
Fig. 1 Flow Chart of the study

Abbreviations: BMS: burning mouth syndrome; BDI: Beck’s Depression Inventory; STAI: State-Trait Anxiety Inventory; VAS: visual analogue scale; SF-MPQ: Short-form McGill Pain Questionnaire; PD-Q: PainDETECT Questionnaire; BPI: Brief Pain Intensity; PSQI: Pittsburgh Sleep Quality Index; ESS: Epworth Sleepiness Scale; SF-36: 36 items Short Form Survey; OHIP-14: Oral Health Impact Profile.
Endodontic Microbiome and its Association With Systemic Inflammatory Burden.
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Objectives Apical periodontitis in endodontic disease is inflammation affecting periapical area of tooth. It can contribute to a low-grade systemic inflammation which might impact patient’s systemic health. This study is aimed to characterize endodontic microbiome and identify its associations with systemic biomarkers profile.

Methods Participants were recruited [(n=35 Re-RCT Group), (n=50 healthy controls)]. Preoperative whole blood and serum samples were collected from control and Re-RCT Group and Intraoperative intracanal samples were collected from the Re-RCT group. Microbiome of blood and intracanal samples were investigated by targeted partial 16S rRNA gene-sequence analysis (V1-V2 hypervariable region) using illumine-MiSeq300 platform. Serum biomarkers profile of 15 analytes were investigated using Multiplex-microbead immunoassay. Microbiome analysis was carried out using DADA2 (version 1.2), R (version 4.1) and Human Oral Microbiome Database (HOMD) (version 15.1). Non-linear association was assessed using Spearman’s correlation and linear associations using Pearson’s correlation. Regression models were used.

Results The prevalent genera isolated from Pre-operative blood samples included Cutibacterium, Staphylococcus, Corynebacterium, Bacillus, Streptococcus, Acinetobacter, Afipia, Sphingomonas, Novosphingibium, Prevotella, Capnocytophaga, Pseudomonas, Mycobacterium, Actinomyces, and Lactobacillus. The prevalent genera identified from the intracanal samples included Enterococcus, Streptococcus, Bacteroidaceae, Lactobacillus, Bacillus, Prevotella, Actinomyces, Tannerella, Flavibacterium, Pseudomonas, Bacteroides, Pseudopropionibacterium, Fusobacterium, Afipia, Parvimonas, Porphyromonas, Dialister, Enterobacter, and Olsenella. The serum biomarkers profile comparison showed significant elevation in the serum levels of IL1b, CRP, FGF-23 and ADMA in Re-RCT group as compared to control group. Positive associations were present between blood Corynebacterium and ICAM1, and Novosphingibium and FGF23. Significant positive association were present between intracanal Bacteroidaceae (G-1) and ADMA, Parvimonas and ADMA and Enterobacter and FGF23.

Conclusions The study results provide evidence of association of endodontic disease and inflammatory markers which may adversely impact patient’s systemic health.
Effect of Sterilisation Techniques on Endodontic Sealers
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Objectives This study aims to analyse the influence of sterilization techniques on the microstructure and chemical properties of endodontic sealers prior to antimicrobial testing. Initial microbial contamination on the material was also assessed.

Methods The materials investigated were AH Plus, BioRoot RCS, BioRoot Flow and TotalFill. The materials were either prepared in sterile conditions in laminar flow chamber or in open lab. Each group was further divided into 5 groups based on type of sterilization technique used to sterilize the sealer. The sterilization methods included ethanol (10 minutes), ultraviolet light (1 hour), ethanol (10 minutes) followed by ultraviolet light (1 hour), autoclave and no sterilization (control). The materials were characterized by scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) and Fourier transform infrared (FT-IR) spectroscopy before and after sterilization, to assess any changes in microstructure and chemical composition. Microbial levels in the materials were assessed by plate streaking technique.

Results Microstructurally, autoclave and ethanol caused deterioration to the material surface of AH Plus. Furthermore, some surface deposits were seen when BioRoot RCS was autoclaved. Ultraviolet increased porosity of the material while ethanol plus ultraviolet seemed to be more destructive to the surface of BioRoot RCS. BioRoot Flow showed some cracking and increased porosity with ethanol plus ultraviolet. TotalFill depicted no major changes.

Chemically, AH Plus and BioRoot RCS depicted changes in water and alcohol peaks whereas TotalFill and BioRoot Flow appeared to be unaffected by sterilization.

All the materials exhibited no contamination when prepared in laminar flow chamber in sterile conditions as compared to sealers prepared in open lab.

Conclusions Different sterilization techniques affected the microstructure and chemical composition of the materials under investigation. It is recommended to check material sterility prior to use and if sterile, avoid sterilization techniques, as these may affect the material properties.

Endodontic Operative Field Asepsis in General Dental Care
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Objectives Studies on endodontic operative field asepsis have generally been carried out by endodontists at specialist clinics. The aim of this study was to evaluate the establishment of an aseptic endodontic operative field in general dental care by assessing general dental practitioners’ ability to reduce the amount of contamination to a non-cultivable level.

Methods 120 adult patients, scheduled for root canal treatment at a general dental care clinic, contributed with 151 teeth. After rubber dam isolation, the operative fields were disinfected with the dentists’ preferred choice of 30% hydrogen peroxide followed by either 5% iodine tincture or 0,5% chlorhexidine-solution. After inactivation (5% sodium thioglycolate/polysorbate 80), samples were taken from the contact area between rubber dam/tooth and occlusal/incisal area. Samples were placed in fluid thioglycolate medium, incubated (37°, 2-5d), evaluated for growth/non-growth, and statistically analyzed (Pearson’s chi-squared test, p<.05).

Results 41.7% (63/151) of the operative fields showed growth. 20.5% (31/151) showed growth from both sample sites. Significantly more positive samples were taken from the contact area than from the occlusal area (p<.001). There were significantly more positive samples when the chlorhexidine-protocol had been used, as compared to the iodine-protocol (p<.001).

Conclusions A sizable proportion of the operative fields showed signs of contamination, indicating incomplete asepsis. It is likely that inadequate isolation of the teeth contributed to the contamination of the operative fields since more positive samples were collected at the contact area than occlusally. The difference between the chlorhexidine- and iodine-protocols may not reflect a true difference in the effectiveness of the antimicrobial solutions, as it could be a consequence of different operators’ skills and thoroughness when isolating and disinfecting the operative field.
O100
Langerhans Cells Orchestrate Initial Establishment of Oral Mucosal Immunity Postnatally
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Objectives We have recently demonstrated the unique neonatal mechanisms induced by the permeable oral epithelium to protect it from bacterial invasion until weaning, a period when the epithelium seals and matures. It is unclear, however, how oral homeostasis is first established after birth. Here, we examined the role of Langerhans cells (LCs), the exclusive antigen-presenting cells of the oral epithelium, in this essential early-life process.

Methods Langerin-DTR mice were used to deplete LCs in vivo (n=5-10 mice), and flow cytometry and RT-PCR analyses were employed to examine oral immunity. The evaluation of the alveolar bone was performed by micro-computed tomography (µCT) and tartrate-resistant acid phosphatase (TRAP) staining.

Results Longitudinal postnatal flow cytometry analysis revealed that LCs populate the epithelium mainly during weaning. These newly differentiated LCs efficiently migrated to the lymph node, the site where adaptive immunity is initiated. In fact, higher percentages of LCs were migrated to the lymph node during weaning compared to adulthood. Conditional depletion of LCs at the weaning period resulted in reduced frequencies of FOXP3+ T regulatory cells in the adult gingiva. Further analysis revealed that LC ablation reduced the expression of pro-inflammatory cytokines such as TNF-α and CXCL1, the latter known to mediate neutrophil recruitment. Consequently, the frequencies of neutrophils and monocytes decreased in the gingiva of adult mice due to the depletion of LCs. The quantification of the alveolar bone in adult mice demonstrated a larger bone volume in LC-ablated mice, which was explained by lower numbers of osteoclasts.

Conclusions Our findings propose a fundamental role of LCs during weaning that control the initial establishment of oral mucosal homeostasis in adulthood. This LC-mediated gingival immunity can nevertheless cause bone loss, exemplifying the nature of this delicate immunological balance characterized as spontaneous bone loss.

O101
Microbial and Osteoblast Responses to Copper-Containing Titanium Implant Material
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Objectives Dental implants are susceptible to failure due to bacterial infection, leading to poor osteo-integration. The aim of this study was to characterise the antimicrobial and osteogenic responses with a titanium alloy containing 5% Cu or titanium alone at the molecular level.

Methods Expression of Cu-sensing transcriptional repressor (csoR), superoxide dismutase (sod) and heat shock protein (Hsp) genes were measured using qPCR in Streptococcus oralis. Global RNA expression profiles of the dental implant pathogens S. oralis, A. naeslundii and Neisseria mucosa were explored via RNA-seq. Expression of Cu transporter and pro-osteogenic genes were examined using immunoblotting and qPCR in MG-63 osteoblast-like cells.

Results In the presence of Cu, significant microbial and osteoblast responses were observed as follows: 1. Expression of csoR, sod and Hsp from S. oralis was enhanced. 2. A. naeslundii transcripts for DNA-recombinase, DUF350 domain containing membrane protein and a HTH ArsR-Type transcriptional repressor involved in stress response to heavy metal ions were differentially expressed. 3. Immunoblotting of the osteoblast cell line revealed that CTR1 (Cu influx transporter), ATP7B (Cu excretion), ATOX1 (intermediate Cu transporter) and LOX (Cu dependent lysyl oxidase) proteins were upregulated. 4. Increased transcription of the pro-osteogenic genes RUNX2, ALP, ON, BMP2, BMP4 and the Cu transporting genes was observed.

Conclusions Copper-containing titanium was shown not only to be detrimental to dental implant pathogens but also offers potentially beneficial osteogenic properties.
Cryotherapy for Managing Oral Biofilms: Beyond Biofilms Removal
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Objectives Cryotherapy is used in dental practice as a safe, easy to perform, and relatively inexpensive treatment for various oral lesions. It is well-known for improving the healing process. However, its effect on oral biofilms is not known yet. Therefore, this study aimed at evaluating the consequences of applying cryotherapy to in-vitro oral biofilms.

Methods Multispecies oral biofilms (14 species) were grown on the surface of hydroxyapatite discs. The formed biofilms were treated with CryoPen® X+ using the appropriate applicator, for 1, 2, or 3 freeze/thaw cycles (5 seconds freeze, with 10 seconds thaw). Biofilms without treatment served as control. Biofilms were split into two groups, one for collection and analysis directly after the cryotherapy, and the other for reincubation for 24 hr to allow recovery of the biofilms. Biofilms then were analysed with qPCR analysis to check the biofilm community compositional changes. The structures of the biofilms after the cryotherapy and 24 hr after recovery from the treatment were studied using a scanning electron microscope (SEM), to follow the possible changes.

Results Cryotreatment of the in-vitro oral multispecies biofilms resulted in a decrease in the biofilm bacterial load, which increased with more treatment cycles. The qPCR results showed that the ecology of the recovered biofilms was significantly better than the non-treated ones. Also, the SEM images of the biofilms showed changes in the layers of the biofilms after applying the cryotherapy, with a dominance of the commensal species in the structure of the biofilms after recovery from the cryotherapy.

Conclusions Cryotherapy might represent a totally new approach in oral biofilm management, that shows promising results as a selective method for eradicating pathobionts and maintaining oral commensals, without using antiseptics or antimicrobials.

Harnessing Natural Competence in Streptococcus Ferus for Oral Health.
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Objectives The ability to take up and incorporate foreign DNA is a well-known characteristic of naturally transformable Streptococci. The objectives of this work were to identify if the understudied species Streptococcus ferus was also capable of natural transformation, using a system similar to that identified in Streptococcus mutans. S. mutans natural transformation is under the control of the alternative sigma factor sigX (also known as comX), whose expression is induced by two types of peptide signals: CSP (compeptid stimulating peptide, encoded by comC) and XIP (sigX-inducing peptide, encoded by comS). These systems control competence via either the two-component signal-transduction system ComDE or the transcriptional regulator ComR, respectively.

Methods Protein and nucleotide homology searches were used to determine if S. ferus encoded for putative orthologs of comRS and/or comCDE. Synthetic peptides encoding for competence-inducing peptide (XIP) from S. mutans or a putative XIP from S. ferus were added to cultures of S. ferus alongside DNA to determine if S. ferus was able to induce natural competence in response to XIPs. Knockouts of genes identified to be important for S. ferus natural transformation were constructed and assessed for their role in natural transformation. Resulting knockout strains were assessed for biofilm formation and growth defects.

Results Protein and nucleotide homology searches identified in S. ferus putative orthologs of comRS, but not comCDE. We demonstrate that natural transformation in S. ferus is induced by a small, double-tryptophan containing competence-inducing peptide (XIP), akin to that of S. mutans, and requires the presence of the comR homolog for efficient transformation. Additionally, we find that natural transformation is induced in S. ferus by both the native XIP and the XIP variant of S. mutans.

Conclusions S. ferus possesses a system for natural transformation similar to that identified in S. mutans. Natural transformation induction in S. ferus by both the native XIP and the XIP variant of S. mutans implies that crosstalk between the two species is possible. This process has been harnessed to construct gene deletions in S. ferus and used to examine the production and biological relevance of tryglysin A, an antimicrobial RaS-RiPP produced by S. ferus.
Mechanical and Physical Properties of Three Bioactive Restorative Materials

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Objectives To evaluate mechanical and physical properties of a new commercially available bioactive restorative material and compare it to established bioactive materials.

Methods Three materials: Cention Forte (CF)(Ivoclar Vivadent), Surefil one (SO)(Denstply), and Riva SC (RSC) high viscosity glass ionomer (SDI) as a control were characterized. Samples were evaluated in terms of fracture toughness, flexural strength, flexural modulus (ISO 4049), Compressive strength and Vickers hardness. Samples were analyzed under a scanning electron microscopy (SEM). Thermogravimetric analysis (TGA) were performed on the three materials, as well as pH measurement of samples in distilled water at times 0, 7, 14 and 21 days. Finally, the sealing ability was evaluated using silver nitrate dye penetration on natural teeth. The data were statistically analyzed using the Kruskal-Wallis test, and one-way ANOVA with Bonferroni post-hoc test, p < 0.05.

Results CF had significantly higher fracture toughness values compared to SO and RSC (p=0.001). Also the FS results showed that CF had significantly higher values (90.11 MPa), followed by SO (22.15 MPa) and then RSC (19.42 MPa), the compressive strength values showed the same order with significantly higher values for CF (231.79 MPa), the flexural modulus and Vickers hardness showed the reverse order with RSC and SO having significantly higher values than CF. pH measurement showed also that CF evolved towards significantly higher pH values at 3 weeks period in distilled water, while thermal properties showed more stability and higher resistance to degradation for the CF compared to the other two. Finally, the silver nitrate penetration results showed significantly better sealing ability for CF compared to the self-adhesive SO and the RSC.

Conclusions Cention Forte showed better mechanical properties except for hardness and better interface sealing ability. Other experimentations should be realized to validate the bioactivity in order to adapt clinical indications according to the results obtained.
Evaluating Cytotoxic Effects and Cytotoxicity Origins of Bulk-Fill Materials by LC-MS/MS

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Objectives To evaluate cytotoxic effects of bulk-fill materials on fibroblast cells and determine cytotoxicity origins by Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) method.

Methods Two dual-cure bulk-fill [HyperFill Dual-cure Bulk-fill (HDC), Parkell, USA; Activa Bioactive Restorative (ABR), Pulpdent, USA], one flowable bulk-fill [Estelite Bulk Fill Flow (EBF), Tokuyama, Japan] materials were used. 4mm depth-5mm diameter plastic rings were filled with non-polymerized materials (n=3). Just before experiment, medium covering cells were removed then rings were placed in direct contact with cells cultured one day prior. Materials were polymerized with light-curing unit (D-Light Pro, GC, Belgium). Polymerization was carried out directly under cell culture conditions. End of polymerization, previously removed medium was re-added to cells in each well. Cells left without medium during preparation of experimental groups (WOM) and cells exposed to light-curing during polymerization of experimental groups (CLCU) were positive control groups. Cells without any treatment were negative control group (C). On 1st, 3rd, and 15th days, cytotoxic effects of materials were evaluated by sulforhodamine B test and cell viability was compared with control groups. End of 1st, 3rd and 15th days, monomers released into the collected medium were determined by LC-MS/MS method based on monomer masses available in literature. Results were expressed as percentages (%).

Results EBF was the most biocompatible material with highest cell viability while ABR showed the most cytotoxic effect with lowest cell viability. Bis-GMA in EBF was absent on 1st and 3rd days while detected on 15th days. Bis-GMA never detected in ABR and HDC. TEGDMA was present in all materials for all time periods. UDMA was present in EBF only on 1st day while was present in ABR for all time periods. UDMA and MAA were not detected in HDC. TEG was detected in every material for all time periods. GMA was present in EBF for all time periods, while was not present in ABR and HDC. MEHQ and Bis-EMA were detected in EBF for all time periods.

Conclusions Since detailed compositions of materials are not shared, it can be thought that the cytotoxicity of the bulk-fill materials may originate from the actual non-shared contents.
**O262**

**A New Calcium Silicate-Based Endodontic Sealer: Angiogenic and Regeneration Potentials**

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**Objectives** The major aim of endodontic therapy is to provide a hermetic apical seal and to achieve a periapical healing which is dependent on the sealer interactions with surrounding tissues. This work was designed to investigate the angiogenic and regeneration potentials of BioRoot™ Flow, a new ready-to-use calcium silicate-based sealer as compared to two currently used materials.

**Methods** Three root canal sealers: BioRoot™ Flow (BRF), Pulp Canal Sealer™ (PCS) and AH Plus® (AH) were prepared and incubated in MEM medium (0.2mg/mL). Human periodontal ligament (hPDL) cells prepared from extracted third molars were incubated with Lipopolysaccharides (LPS) ± sealers’ extracts. Their proliferation and colonization were evaluated using MTT and the scratch assay. The secretion of growth factors (VEGF, FGF-2 and TGF-β1) and Complement C5a was quantified by ELISA. The effect of hPDL supernatants on human Bone Marrow Stem Cell (hBMSC) migration was investigated using Boyden chambers. Its effect on endothelial cell (HUVEC) proliferation was investigated with the MTT assay while its neo-angiogenenic potential was evaluated on Matrigel.

**Results** BRF significantly increased TGF-β1, VEGF, FGF-2 and C5a production. An increase of VEGF, FGF-2 and C5a was also obtained with AH but to a lesser extent than with BRF. In addition, both significantly induced hPDL proliferation and colonization as compared to the control and PCS. However, a higher increase was observed with BRF. Moreover, BRF and AH increased endothelial cell proliferation and induced a C5a-dependant hBMSC recruitment. Finally, a decrease in endothelial cell organization into tube-like structures was observed only with AH and PCS.

**Conclusions** This work highlights that endodontic sealers modulate the target tissues regenerative and angiogenic potentials. This suggests that, in addition to its adequate use in endodontic procedures, the sealer’s interaction with the surrounding tissue is of prime importance for periapical healing.

**O263**

**M2c Polarization of Inflammatory Monocytes (iMos) Mediated by Nanostructured Implant**

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**Objectives** Although it has been established that macrophage M1/M2 polarization can be manipulated by the surface nanostructure of biomaterial in our previous study, the detailed subtype of the M2 (M2a, b, or c) macrophages derived from specific subsets of human peripheral monocytes remains unclear. The role of the cytoskeleton rearrangement in the stress perception of macrophages, especially in the regulation of macrophage polarization under weightlessness, needs to be further clarified as well.

**Methods** In the present study, we purified human peripheral CD14+CD16- inflammatory monocytes (iMos) and investigated their inflammatory differentiation on nanostructured Ti implant surfaces (NTs) using a set of analytical methods. According to our hypothesis that cytoskeleton is required for cells to sense the extracellular topographical/stress signals, cytochalasin D treatment was performed in this study to clarify the necessity of intact cytoskeleton rearrangement function in macrophage polarization on biomaterials surface.

**Results** iMos were directly induced into M2c or M1 phenotype on NTs surfaces with specific nanotube sizes (~30nm or ~80nm respectively) without exogenous inducers. Cytochalasin D treatment greatly abrogated the manipulative effects of NTs surfaces on macrophage polarization with both tube sizes, most likely due to the crucial role of cytoskeleton rearrangement in macrophage polarization on biomaterials.

**Conclusions** This is the first metallic implantable materials study focusing on the functions of specific monocytes subset, which enriches our mechanism knowledge of the crosstalk between immunocytes and biomaterials. The limited cytoskeletal rearrangement function provided a possible explanation for the hampered bone formation around Ti implantable devices. The results obtained in the present study may also provide potential interventional targets for improving the design and modification of implantable materials via immune precise regulation, especially in certain circumstances.
Apatite Formulation Detection With an X-ray Photoelectron Spectroscopy
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Objectives Implant surfaces are often modified by a bioactive glass coating layer to stimulate apatite formulation promoting bone growth and for improved osseointegration. The time-dependent apatite formation of bioactive glasses is commonly characterized by FTIR, XRD and solid-state NMR. This study aims to explore the effectiveness of XPS in characterizing the change of the chemical state of bioactive glasses and apatite formation.

Methods Rectangular silicon wafers (approximately 10*10 mm², n=14) were prepared; ultrasonically cleaned in ethanol, rinsed with deionized water and used as reaction substrate. 45S5 bioglass (D50 of 51 microns) was dispersed into ethanol (10 times of glass powders in volume) and ultrasonicated for 10 mins. After sedimentation, the top liquid layer was extracted. The resultant suspension was dropped onto silicon wafers. Samples were then subjected to immersion in SBF solution in an incubator (1 min, 2 mins, 30 mins, 2 h, 8 h, 1 d and 4 d). After removal, samples were rinsed and investigated using X-ray photoelectron spectrometer with an Al Kα X-ray source and a monochromator.

Results The XPS analysis shows the presence of calcium peaks correlating to Ca in the glass for samples with 1 and 2 mins SBF immersion. The characteristic calcium peaks for hydroxyapatite were noticed for samples subjected to 30 mins and longer SBF immersion. The proportion of characteristic calcium peaks for hydroxyapatite was found to increase with an increase in the immersion time.

Conclusions The chemical state changes of calcium and apatite formation were clearly identified and monitored by XPS. The presence of apatite was observed as early as 30 mins SBF immersion, indicating that XPS is a highly sensitive technique for the detection of apatite formation.

Periodontal Disease Prevalence and Oral Hygiene Practices in Adults With Cystic Fibrosis
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Objectives Cystic Fibrosis is a life-limiting condition and is the most common autosomal recessive disease in Caucasians. A study was conducted to investigate the oral hygiene status, and the prevalence and severity of periodontal disease in adults with Cystic Fibrosis in the Republic of Ireland.

Methods 100 subjects were recruited to the study. Inclusion criteria included being aged 18 or over and having a diagnosis of Cystic Fibrosis. An oral health self-assessment was completed by participants and a clinical intraoral examination was carried out by calibrated (ICC=.94) dentists. Oral Hygiene was assessed using the WHO-recommended Greene-Vermillion Oral Hygiene Index, measuring the amount of plaque and calculus present on the teeth. Periodontal health was measured using the Community Periodontal Index (CPI). Subjects with a periodontal pocket depth of 4mm or more were classified as having periodontal disease.

Results A total of 100 patients with Cystic Fibrosis took part in the study, 60 were male, 40 were female. The median age was 30.5 (minimum age 18, maximum age 69). 71% brushed twice or more daily, 17% reported brushing once daily, 9% brushed less than once daily. 54% occasionally or regularly use mouthwash. 50% never use interdental cleaning aids. 95% were non-smokers or never smoked. 32% reported attending the dentist or dental hygienist for regular dental cleaning. 50% of respondents had not attended the dentist in the past year. The mean (SD) debris index was 0.1 ± .13 (out of a maximum of 6). The mean (SD) calculus index was 0.6±.77 (out of a maximum of 6). The mean (SD) Oral Hygiene Index was .7 ± 0.9 (out of a maximum of 12) Participants had a mean(SD) of 7.9 ± 7.6 bleeding sites per mouth. 15% of participants were classified as having mild periodontal disease, with PPD of 4-5mm. No participant was classified as having severe periodontal disease.

Conclusions Adults with Cystic Fibrosis had very low levels of debris and calculus. Their oral hygiene regime was generally good, although 50% do not use interdental cleaning aids. The sample studied had low levels of gingivitis and periodontal disease. It is possible that Cystic Fibrosis, or the drug regimens (particularly antibiotics) employed in its management have a preventative effect on periodontal disease.
Radiological Evaluation of Prevalence of Bone Loss and Teeth Grinding

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Objectives
This study evaluated the prevalence of alveolar bone loss and tooth wear through periapical radiographs.

Methods
This retrospective analysis included 1,950 series of periapical x-rays. For statistical evaluation, only teeth 41 and 33 were examined, and the following data were evaluated:
(1) Extent of marginal alveolar bone loss applying Schei’s ruler method.
(2) Radiographic tooth wear was examined in the two mandibular incisors and categorized by the extent of enamel loss.

Results
1,400 subjects met the inclusion criteria. In all age groups, a statistically significant correlation was found between marginal bone loss and tooth wear in teeth 33 and 41, with a correlation to age and gender. In all age groups, tooth wear was significantly related to bone loss severity (p<0.001). The masking effect of age on bone loss and tooth wear was eliminated by logistic regression analysis. The odds ratio of having tooth wear in patients with bone loss was 2.004 and 2.576 for teeth 33 and 41, respectively. PDL widening was statistically significantly correlated to bone loss and tooth wear (p<0.0001).

Conclusions
In the present study, a statistically significant non-age-dependent correlation between tooth wear bone loss and PDL widening was found.

Figure 1: Frequency of bone loss, tooth wear, and PDL widening (percentages)

Figure 2: Mean age of patients with different degrees of periodontal and teeth pathologies

Figure 3: Correlation of PDLw of teeth 33 and 41, tooth wear, and bone loss in teeth 33 and 41.
The Physical Activity Paradox and Periodontitis: a Population-Based Study
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Objectives Evidence from the medical field has shown how leisure time and occupational physical activity (PA) exert an opposite effect on the incidence of several chronic non-communicable diseases, and this phenomenon has been described as the ‘physical activity paradox’. The aim of the present study was evaluate the association between recreational/occupational PA and periodontitis in a nationally representative sample of the US population.

Methods Data from 10,679 adults (≥30 years old), representative of 431.1 million people, were retrieved from the NHANES 2009-2014 database. PA was assessed using the World Health Organization validated questionnaire, which analyzes the frequency of recreational and occupational PA during a typical week; subjects were consequently classified as performing either high or low recreational and occupational PA. A full-mouth periodontal examination was performed to identify periodontitis and to classify its severity according to the AAP/CDC criteria (mild, moderate, severe). Univariate and multivariate regression analyses were applied to study the association between recreational/occupational PA and periodontitis/severe periodontitis.

Results Multivariate analyses identified high recreational PA as protective indicator for periodontitis (OR=0.81), while high occupational PA resulted as a significant risk indicator (OR=1.21). The combination of low recreational/high occupational PA showed a multiplicative independent association with periodontitis (OR=1.46). Moreover, both high recreational PA (OR=0.66) and occupational (OR=1.47) resulted independently associated with stronger estimates of severe periodontitis, and the same was observed for the combination of low recreational/high occupational PA (OR=2.07). These independent associations resulted partially mediated by systemic inflammation, BMI, diabetes and hypertension. The sensitivity analyses also demonstrated a dose-response relationship between both types of PA and periodontitis/severe periodontitis.

Conclusions The results from the present population-based study indicate the presence of a physical activity paradox also on periodontitis, since high recreational PA resulted as a protective indicator and high occupational PA as a risk indicator of the disease, with both multiplicative and a dose-response type relationships.

Tooth Loss and All-Cause Mortality: a 19-Year Prospective Cohort Study
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Objectives The aim was to investigate the association between tooth loss and all-cause mortality in a prospective study of a cohort of men aged 58–72 years.

Methods A representative sample of 1558 men in Northern Ireland had a detailed dental examination between 2001 and 2003. The primary end point was death from any cause. The men were divided into groups by the number of teeth they had at the baseline examination: high (≥20); medium (10-19); low (1-9) and edentulous.

Multivariable analysis was carried out using logistic regression with adjustment for possible confounders to calculate odds ratios (OR) and 95% confidence intervals (CI).

Results The mean age of the men at baseline was 64.3 (SD 2.9) years. In total, 599 (38.4%) of the men died during a mean follow-up of 18.5 (SD 0.7) years: 257 (31.7%) in the high number of teeth group died compared with 178 (39.4%) in the medium; 69 (50.7%) in the low; and 95 (60.1%) who were edentulous. After adjustment for confounding variables (age, smoking, BMI, C-reactive protein, diabetes, education, and socio-economic status) the OR (95% CI) relative to those who were in the high teeth group were 1.19 (0.91–1.54), p = 0.21 for the medium; 1.68 (1.13–2.50), p = 0.011 for the low; and 2.22 (1.50–3.30), p <0.0001 for the edentulous group. A test for trend across categories was significant, p<0.01.

Conclusions There was evidence in the Western European men studied that those who were edentulous or had less than 10 teeth had a significantly increased risk of all-cause mortality.
Objectives The aim of this study was to assess the oral and nasal microbiome signatures of patients with COVID-19, with the goal of gaining a better understanding of the interplay between microbial communities and ultimately aid with the management of viral upper respiratory illnesses.

Methods An observational cohort study of 102 patients with COVID-19 (51 mild and 51 severe) and 50 controls was conducted at University College London Hospital, UK between December 2020 and June 2021. Mild COVID-19 cases were recruited among hospital-ward based patients, while severe cases were undergoing care in the intensive care unit. Controls were generally healthy COVID-19 negative individuals. Swab samples from the mouth and anterior nares were collected at one time point, DNA was extracted, and 16S rRNA amplicon sequencing of the DNA was performed on the Illumina platform.

Results Alpha diversity of COVID-19 patients with severe disease was significantly different (lower) than that of controls and mild for oral samples. Beta diversity from the nasal swabs of severe COVID-19 patients was statistically significantly different from the same swabs of patients with mild symptoms and controls (PERMANOVA, P=0.001), whereas in the beta diversity analysis of oral samples each study group clustered significantly from each other and differed significantly (PERMANOVA, P<0.001). Specific genera, including Pseudomonas, were identified as potential microbiome signatures associated with more severe COVID-19 disease.

Conclusions Oral and nasal microbiomes unique signatures were identified in patients with COVID-19 with oral samples demonstrating most distinct differences across control, mild and severe cases. These data, when combined with other oral, nasal, and gut microbiome sequencing data from COVID-19 patients, may allow for better patient stratification and management of patients with respiratory viral infections, particularly COVID-19.
Effects of Poly-Gamma-Glutamic Acid Produced From Different Sources on Demineralisation Inhibition of Hydroxyapatite

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Objectives Poly-gamma-glutamic acid (gPGA) is a low-cost Bacillus-derived material with a great potential for a range of applications, from tuneable drug delivery system to enamel demineralisation inhibitor. The salivary protein statherin protects tooth enamel from acid dissolution and acts as a reservoir for free calcium ions within oral cavity. Its superb enamel-binding capacity is attributed to the L-glutamic acid residues of this 5380 Da protein, which has shown to reduce demineralisation in caries-simulating model systems. As such, bioderived gPGA holds promise as a good model system for emulating statherin. After production, gPGA can be purified by variety of methods, including tangential flow, a form of molecular weight selective dialysis. The aim was to measure the cariostatic efficacy of gPGAs produced from different sources and treatments, on the demineralisation inhibition of hydroxyapatite (HAP) exposed to caries simulating conditions.

Methods gPGA formulations were obtained from: commercial suppliers (700 KDa), algal biomass but no tangential flow purification (MGP21-008-R2), algal biomass purified with tangential flow (MGP21-008-R3_PTF), hydrolytically degraded commercial samples (MGP21-067-B&C), microalgal biomass + waste brewery yeast but no tangential flow (MGP21-054-R2), pre-extracted microalgae biomass purified with tangential flow (MGP21-047(44)_PTF).

HAP discs (20% porosity) were immersed in deionised water. Each gPGA formulation was prepared at 1 w/v% concentration in distilled water. Each disc was treated with one gPGA formulation for 5 mins, as was an untreated control, then exposed to acetic acid (pH4.0) for 1h. Real-Time Ion Selective Electrodes (RTISEs) were used to measure calcium ion release (as a proxy for demineralisation) every 60s throughout. The percentage demineralisation inhibition was calculated for each gPGA treatment.

Results All gPGA tested decreased HAP demineralisation by over 90%, except the macroalgae derived gPGA with no tangential flow purification.

Conclusions gPGAs from different sources are highly effective inhibitors of HAP demineralisation in caries simulating conditions. Purification is crucial for cariostatic efficacy.

Systematic Review and Meta-Analysis on Physical Barriers to Prevent Dentin Demineralization

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Objectives The present review systematically analyzed in vitro and in situ studies investigating physical diffusion barriers (sealants, desensitizer or adhesives) to prevent the initiation or the progression of dentin caries lesions.

Methods Three electronic databases (PubMed-Medline, CENTRAL, Ovid-EMBASE) were screened for studies from 1946 to 2020. Cross-referencing was used to identify further articles. Article selection and data abstraction were done in duplicate. Languages were not restricted. Outcomes were lesion depth, penetration depth, dentinal tubule occlusion and antibacterial activity. For all outcomes mean differences (MD) were calculated using fixed- or random-effects models.

Results From 167 potentially eligible studies, 34 studies evaluating 69 different materials were selected for full text analysis and 17 studies – still evaluating 36 different materials – were included (3 in situ and 14 in vitro). Ten studies evaluated desensitizers, 8 adhesives and 1 infiltration. Meta-analyses were possible for all 17 studies. Meta-analyses revealed that lesion depth after no treatment was significantly higher than after the application of single-step adhesives (MD[95%CI] =-49.82 [-69.34;-30.30]) and multi-step adhesives (MD[95%CI] =-64.88 [-95.31;-34.46]). No significant differences in the lesion depth increase between single- and multi-step adhesive could be observed (MD[95%CI] =25.21 [-20.34;70.77]). Furthermore, compared to no treatment the increase of the lesion depth was significantly hampered using desensitizers (MD[95%CI] =-38.02 [-51.74;-24.31]).

Conclusions A physical diffusion barrier can significantly hamper the lesion depth increase under cariogenic conditions. Furthermore, multi-step adhesives seem not to be more effective than single-step adhesives. However, this conclusion is based on only very few in vitro and in situ studies.
O113
Efficacy of CPP-ACP on Early Caries Lesions Remineralization: a Meta-Analysis
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Objectives There is a growing need for effective methods for the management of early-stage caries lesions. Therefore, we aimed to evaluate the efficacy of combined fluoride and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) treatment on remineralizing white spot lesions (WSLs) compared to fluoride-only interventions.
Methods This meta-analysis was performed according to PRISMA guidelines. Medline, EMBASE, Cochrane Central databases were searched up until February 2022. Eligible studies were Randomized Controlled Trials (RCTs) written in English. Outcome variables included Laser Fluorescence (LF), Quantitative Light-Induced Fluorescence (QLF) and lesion area scores. The random effects model was used and results were analyzed as standardized mean difference (SMD) and mean difference (MD) with 95% Confidence Interval. Risk of Bias was assessed with RoB 2 tool and level of evidence with GRADE.
Results Our systematic search yielded 867 records after duplicate removal, 21 studies included for qualitative synthesis and 15 studies were eligible for quantitative analysis. No significant difference between CPP-ACP and fluorides versus fluorides alone were found by LF at 1, 3 and 6-month use (SMD -0.40 [-0.75; -0.06]; SMD -0.59 [-1.20; 0.03]; SMD -0.46 [-1.11; 0.19], respectively). For QLF, the analysis did not demonstrate significant differences between these two treatments at 1 and 6-month use (MD 0.21 [-0.30; 0.71]), but at 3-month, a superior remineralization in the fluoride-only group over the CPP-ACP and fluorides was shown regarding the white spots (MD 0.58 [0.25; 0.91]). On the contrary, a significant decrease was seen in the lesion area favoring the CPP-ACP and fluorides vs fluoride alone, at six months (MD -0.38 [-0.72; -0.04]). None of the changes indicated substantial clinical relevance.
Conclusions The combination of CPP-ACP and fluorides did not overcome fluoride-only results. But reinforcement of daily oral hygiene using fluoridated toothpaste achieved remineralization of WSLs. Development of better products are needed to achieve robust beneficial effects on WSLs.

O114
Synergistic Influence of Strontium and Fluoride on Hydroxyapatite Demineralisation
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Objectives Strontium and fluoride are thought to be synergistic in preventing caries. The objective of this study is to explore this possible synergy of strontium and fluoride on the demineralisation of hydroxyapatite discs under acidic conditions.
Methods Porous hydroxyapatite (HA) discs were used as an analogue of tooth enamel. The discs were immersed in 0.1M Acetic Acid pH 4.0 in solutions containing 0, 10, 50 and 100ppm Sr2+, F− and Sr2+ and F− combined. The dissolution of the disc was followed by measuring the free Ca2+ ion concentration using an ion selective electrode in real time. The final immersion solutions were analysed for Ca, Sr and P by Inductively coupled optical emission spectroscopy. Finally the discs were analysed by 19F solid state nuclear magnetic resonance spectroscopy (ssNMR) to detect any fluoride containing phases formed.
Results The presence of 50ppm and 100ppm Sr2+ accelerated the Ca2+ release, whilst 10ppm Sr2+ decreased Ca2+ release from the HA. Fluoride in solution decreased the release of Ca2+ from the HA as expected. All three solutions with both Sr2+ and F− decreased the release of Ca2+ from HA more than F− alone. The 19F ssNMR showed the formation of calcium fluoride (CaF2) with 100ppm F− and fluorapatite (Ca5(PO4)3F) at 10ppm F−. Surprisingly there was no evidence for the formation of mixed strontium calcium fluorapatite phases, where both Sr and F were present that has been proposed previously to explain the synergistic action of strontium and fluoride. Furthermore the presence of strontium suppressed both the formation of calcium fluoride and fluorapatite found with fluoride alone.
Conclusions The proposed synergistic mechanism of action of strontium and fluoride in forming mixed calcium/strontium fluoride phases is not supported by ssNMR measurements. A new mechanism of action of strontium and fluoride on demineralisation/remineralisation is required.
Determination of the Remineralizing Effectiveness of Bioactive Dental Varnishes

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Objectives The aim of this study is to compare the remineralization efficiency of fluoride-free varnish combinations containing bioactive glass, eggshell and membrane powder and fluoride varnish formulations on artificial caries lesions on the enamel surface.

Methods Demineralized artificial caries lesions were formed in two windows on third molars. One of the windows on each teeth sample was coated with one of the following varnish formulations: FV (fluoride varnish), F-BAGV (fluoride and bioactive glass containing varnish), BAGV (bioactive glass containing varnish), EPV (eggshell powder containing varnish), EMP-EPV (eggshell membrane protein and eggshell powder containing varnish), STMP-ESPV (sodium trimetaphosphate treated eggshell membrane protein and eggshell powder containing varnish). The uncoated window on each tooth served as demineralized lesion, whereas the coated one used as remineralized area. The samples were investigated under scanning electron microscopy (SEM) and elemental analyses were performed by X-ray dispersive (EDX) analysis. In addition, the traditional colorimetric tetrazolium-based reduction assay (XTT) and the modern impedance-based real-time cell analysis (RTCA) system to investigate their cytotoxicity in vitro.

Results Similar Ca, P, F, O, C, Mg and Na element levels were detected between the demineralized control regions and the varnish-applied regions (P>0.05). The rate in remineralized areas of all groups was lower than stoichiometric hydroxyapatite except ESPV and STMP-ESPV group. The 1/1 dilutions of the all-tested groups were statistically different from the control group. Merely 1/2, 1/4 and 1/8 dilutions in the FV group and 1/2 dilution in the F-BAGV group showed statistically different effects on cell viability than the control group.

Conclusions Bioactive glass, eggshell, eggshell membrane proteins and STMP-treated eggshell membrane might improve remineralization. Also, integration of biological or bioactive components that provide remineralization into the formulations of dental varnishes may reduce cytotoxicity.

Efficacy of Silver-Fluoride Application on Root Caries Using High-Contrast XMT

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Objectives The objective of this study was to compare the efficacy of different topical fluoride applications either containing silver-fluoride (AgF), silver-diamine-fluoride (SDF), or sodium-fluoride (NaF) on root caries (RC) using high-contrast X-ray Microtomography (XMT).

Methods Visual and tactile assessments of 150 teeth were carried out. A total of 8 teeth with RC (severity indices 1 and 2) were selected. All teeth were cleaned and polished using non-fluoridated prophylaxis paste (Nupro Dentsply, USA). Each lesion was examined according to colour, hardness, texture, size, cavitation, and severity. These teeth were randomly assigned to four groups without the removal of lesions: Group 1. 38% AgF (SDI RivaStar Aqua, Australia); Group 2. 38% SDF (SDI RivaStar, Australia), Group 3. 2.26% NaF (Colgate Duraphat, UK); Group 4. Toothbrushing alone. Samples were separately stored in artificial saliva (pH4.8) for 42-days at 37°C. Standardised toothbrushing using 1,450 ppm fluoridated toothpaste (Colgate-Total, UK) was carried out for all samples twice a day. The XMT scans were performed at baseline, immediately after each topical fluoride application, at 21 and 42-days (duration of each scan was up to 24hrs). The XMT images were then reconstructed for each sample to measure the loss/gain of mineral density at demineralised sites.

Results The XMT subtracted images verified an increase in mineral density within RC after 42-days for Groups 1, 2 and 4 (p<0.05). The mean percentage of mineral gain was high in Group 1 (221%, p<0.05) in comparison to Groups 2 and 4 respectively (34%, 56% p<0.05). However, there was no mineral-density change at the demineralised sites for Group 3 (p>0.05).

Conclusions This laboratory-based study demonstrated that XMT is a useful apparatus to quantify the mineral loss/gain within RC. The 38% AgF-application was promising for reversal of leathery-type of RC without the removal of carious lesions when compared to the 38% SDF, 2.26% NaF applications and toothbrushing alone.
O117

Common Genetic Background of Hypodontia and Third Molar Agenesis

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Objectives In this cross-sectional study, we aimed to investigate prevalence patterns of hypodontia and third molar agenesis, to discern whether the association is a spurious one, and to perform a genome-wide association study on third molar agenesis.

Methods We examined dental panoramic radiographs (DPRs) of 4,608 adolescents (2,333 girls and 2,275 boys) with a mean age of 13.62 years, participating in Generation R, a multiethnic population-based cohort based in Rotterdam, the Netherlands. One experienced examiner ascertained hypodontia and third molar agenesis (M3a) separately. Chi-square test was used to establish whether frequency in M3a differed between participants with and without hypodontia. Recall-by-Genotype (RbG) approach was performed on participants genetically determined to be of European ancestry (3,563 adolescents). Weighted polygenic scores (wPGS) were constructed for hypodontia, with the relevancy assumption tested. Using RbG it was established whether hypodontia and M3a shared genetic background. A genome-wide association study (GWAS) was performed against M3a, using an additive genetic model (4,306 adolescents).

Results Hypodontia and M3a prevalence were 4.904% and 13.41%, respectively. Hypodontic adolescents were more likely to have M3a than those without (21.7% vs 13.0%, P-value < 0.001). Mean hypodontia wPGS was 0.914 (standard deviation of 0.465; range 0.000 – 3.520). Each wPGS unit increase conferred 76.1% odds increase for hypodontia (P-value < 0.001). 17.4% of participants in the top quintile of the wPGS distribution had M3a, opposed to 11.9% in the bottom quintile (P-value = 0.004). GWAS uncovered one genome-wide significant genetic variant (rs242214-G, p-value: 4.32x10^-8, odds ratio: 2.792 per effect allele), mapping to FHIT gene.

Conclusions By leveraging genetic methods, we established third molar agenesis and hypodontia share a common genetic background. Genome-wide association study of third molar agenesis uncovered a single significant locus, mapping to novel biology. To our knowledge, we are the first to establish these results.
Enzyme-Responsive Nanoparticles for Dexamethasone Targeted Delivery in Diabetic Rats

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Objectives Diabetes is accompanied by numerous complications, among which impaired wound healing and increased risk of infection that may lead to large incidence of secondary infections and resistance to standard treatments. Thus, there is enormous needs to develop efficient methods to target drugs delivery to the affected tissues. Enzyme-responsive nanoparticles can target inflamed tissues through the enhanced permeability and retention effect and aggregate in response to upregulated matrix-metalloproteinases. Our hypothesis of work is that targeted drug delivery by means of enzyme-responsive nanoparticles may improve treatment efficacy in inflammatory lesions.

Methods The aim of the present study was to demonstrate delivery, accumulation and therapeutic effect of fluorescent dexamethasone-conjugated enzyme-responsive nanoparticles in collagen membranes implanted in the calvaria of diabetic rats. Diabetes results in accelerated degradation of collagen membranes in the rat model, therefore providing a quantitative approach to histologically evaluate the degree of membrane resorption based on the residual collagen in the implanted membranes. Dexamethasone-incorporated nanoparticles were injected adjacent to the area implanted with the biotin-labeled collagen membrane.

Results One-week post-injection, in animals that received nanoparticle treatment, a strong fluorescence was observed around the implanted membrane and not at the site of injection, thus confirming targeted accumulation at the site of inflammation. At 7- and 14-days post injection, the in-vivo fluorescence intensity from enzyme-responsive nanoparticles was significantly higher compared to non-responsive nanoparticles controls. Through avidin-HRP and other staining methods, histologic analysis showed that the remaining collagen membrane area post enzyme-responsive nanoparticles treatment was significantly higher compared to the free dexamethasone group. Hence, incorporation of dexamethasone into the enzyme responsive nanoparticles improves its therapeutic efficacy of reducing inflammation, potentially by increasing local drug concentration and prolonging its circulation half-life.

Conclusions This study demonstrates the potential use of enzyme-responsive nanoparticles as targeted drug delivery method for the treatment of diabetic and other inflammatory wounds.
Novel Engineered Collagenase for Enzymatically Assisted Minimally Invasive Tooth Extraction

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Objectives Minimally invasive exodontia is among a long-sought-for aim for safe dental medicine. Our objective is to design an optimized collagenase by harnessing protein engineering methods. We demonstrated that the injection of wild-type enzyme significantly reduces the force required for tooth extraction. Herein, we aim to inject an optimized engineered collagenase to the periodontal ligament fibers to examine whether this new enzyme further reduces the force required for tooth extraction.

Methods Recombinantly expressed clostridial collagenase G variant purified from Escherichia coli was designed by computational algorithms and injected into the periodontal ligament of mesial and distal roots of the first and second split porcine mandibular premolars. Vehicle solution was injected into the corresponding roots on the contralateral side. Following sixteen hours, the treated mandibles were mounted on a loading machine to measure the extraction force. In addition, the effect of the enzyme on the viability and toxicity of CHO and fibroblast cells was evaluated.

Results Following the injection of recombinantly expressed collagenase harboring the wild-type sequence, an average reduction of 20% in the applied force (albeit with a large variability of 50 to 370 newton) was observed for enzymatically treated roots, reaching up to 50% reduction in some cases. Injection of first-generation optimized collagenase further reduced the force with an average reduction of 15% compared to the wild-type enzyme. Importantly, the enzyme showed only minor, transient cellular toxicity.

Conclusions Using an innovative model enabling analytical measurement of extraction forces, we showed that enzymatic disruption of periodontal ligament fibers substantially reduces the force required for tooth extraction. This novel technique brings us closer to atraumatic exodontia, potentially reducing complications and facilitating subsequent implant placement. Engineering of novel enzymes may further simplify the tooth extraction process and present additional clinical relevance for the broad range of implications in the oral cavity.

Enzymatically driven exodontia

Injection of novel collagenase to the periodontal ligament reduces the force required for tooth extraction
Blood Pressure Screening for Hypertension at Peninsula Dental School, UK
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Objective: An estimated 5.5 million people (8\%) in the UK may have undiagnosed hypertension, presenting higher risk of a major cardiovascular event such as fatal heart attacks or stroke. The dental team could play a vital role detecting undiagnosed hypertension and thus the aim of this pilot study, was to detect undiagnosed hypertension in patients attending at Peninsula Dental School (PDS) in an area of low socioeconomic status.

Methods: New patients (>40 years/old) attending the Devonport Dental Education Facility (PL1 4JZ) were recruited for this study; the clinic being situated in the top 4\% of most deprived areas of England. Participants were invited to return within 4 weeks for a ‘health screening’ clinic, Systolic (SBP) and diastolic (DBP) blood pressures, atrial fibrillation (AF) and BMI were assessed and patients were referred to the GP, and provided with information for self referral to local services, when high values of blood pressure (SBP >140 mmHg; DPB >90 mmHg) were found.

Results: The return rate for screening after recruitment was 71\%. 30 participants were screened with 5 (16.6\%) participants demonstrating SBP >140 mmHg and not receiving hypertension medication (undiagnosed hypertension). Furthermore, 3 (10\%) of patients had a SBP >140mmHg despite being on existing hypertension medication (resistant hypertension). 3 patients (10\%) had a SBP between 130-140mmHg (pre-hypertension). Overall 14 (47\%) of participants were overweight (BMI>25) and 8 (27\%) were obese (BMI>30)

Conclusions: Peninsula Dental School was a successful setting for detecting patients with undiagnosed hypertension. High blood pressure was confirmed by GPs and 18\% of patients with undiagnosed hypertension exceeds previous UK estimates. This may be due to our clinics operating in areas low socioeconomic status. Measuring blood pressures of patients attending dental schools thus has potential to save thousands of lives per year via detection and prevention in at risk populations.
Treatment of Melanin Gingival Pigmentation With Q-Switched Nd:YAG Laser

Nicolò Giuseppe Armogida¹, Elena Calabria², Flavia Iaculli¹, Mariangela Cernera¹, Alessandra Valletta¹, Gianrico Spagnuolo¹

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Objectives: Gingival pigmentations are purplish discolorations or irregularly shaped brown spots or larger areas. These common and probably hereditary conditions are determined by the accumulation of melanin due to the hyperactivity of melanocytes in the basal and supra-basal epithelial layers. To date, no data are available with regards to Q-switched Nd:YAG Laser used for gingival pigmentation.

In order to provide a conservative approach, the off-label application of Laser has been tested, in terms of efficacy and rate of post-operative complications.

Methods: Ten patients (4 male, 6 female, median 38 years) with melanin gingival pigmentation and free of periodontal disease were treated with the Q-switched Nd:YAG laser (Synchro QS4 Deka, M.E.L.A s.r.l., Calenzano (FI), Italy), using non-contact technique.

The following parameters were applied: wavelength of 532 nm, fluence of 2.3 J/cm², frequency of 1 Hz, spot size 2.5 mm. No local anesthesia was required.

Verbal rating score was used to evaluate discomfort.

Results: One to 3 applications were carried out at 3-week intervals, depending on the degree of pigmentation and in accordance to the agreement between 3 independent clinicians.

Picture of the gums were taken at baseline, at the endpoint of the treatment and 3 weeks after the laser application. Then follow up was performed at 6, 12 and 24 months. In all evaluated cases pigmentations were completely removed.

A slight discomfort during normal oral hygiene procedures was reported by one patient (VRS=1) in the first 3 days after the treatment. No oral discomfort after 1, 3 and 7 days (VRS=0) was appreciated in the other 9 patients.

Conclusions: The treatment was proved to be effective without any reported complications or major discomfort, thus laser treatment could be a valid alternative to conventional surgery. However, further studies with a longer follow-up and a wider sample size are needed.
**Molecular Pathways in Burning Mouth Syndrome**

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**Department of Oral Medicine, Sedation & Maxillofacial Imaging, Faculty of Dental Medicine, Hadassah Medical Center, Hebrew University, Jerusalem, Israel, Institute of Biomedical and Oral Research, Faculty of Dental medicine, Hebrew University, Jerusalem, Israel**

**Objectives** Burning mouth syndrome (BMS) is a chronic neuropathic pain condition, characterized by a burning sensation from the oral mucosa, predominantly affecting postmenopausal women. There is no efficient treatment or known molecular pathways involved in the pathogenesis. We aim to identify potential biomarkers and relevant biological pathways, via molecular profiling of tongue epithelial cells, from patients with BMS and healthy individuals - that may serve as a basis for intervention. The secondary objective is to evaluate the clinical and molecular effects of topical tretinoin gel treatment for BMS patients since these molecules affect the pain-related receptor Transient Receptor Potential Vanilloid (TRPV) and its known effect on keratinization.

**Methods** Tissue samples from the lateral dorsum of the tongue were obtained from 12 post-menopausal women, 7 previously diagnosed with BMS. The samples underwent RNA extraction by TRIZOL method, and processing using Affymetrix human mRNA expression chips to determine gene expression profiles. Ten BMS patients participated in a 2-week clinical trial examining the effects of a twice-daily application of topical retinoids, tretinoin gel 0.05%. The clinical evaluation will include demographic data and pain characteristics before and after treatment.

**Results** Initial results showed 73 upregulated genes and 150 downregulated genes in the BMS group. Ten of the most downregulated genes possess a functional relation to keratinization and cornification. Among these 10 are the keratin-6 (KRT6) genes which play a role in epidermal wound healing. Additionally, initial clinical 2-week use of tretinoin gel 0.05% showed a decrease in pain level.

**Conclusions** These initial findings suggest that the downregulation of KRT6 may be related to the BMS mechanism through involvement in the keratinization process. The tretinoin gel 0.05% treatment primary efficacy may be related to this process, since TRPV expression may be altered during keratinization. Further large-scale, investigation is required on a clinical and molecular level.

**Fast Curing Bulk-Fill Resin Composites: a Systematic Review.**

Elisabeth Dursun, Philippe Francois, Jean-Pierre Attal

**URB2i, Université Paris Cité, Montrouge, France, Henri Mondor Hospital, Créteil, France, Bretonneau Hospital, Paris, France, Charles Foix Hospital, Ivry-sur-Seine, France**

**Objectives** Which practitioner has not wished to reduce photopolymerization time? The three second power cure system, consisting in an adhesive, a bulk-fill resin composite and a high-power curing light, is claimed to maintain the reliability of the cure process in only 3s. The aim of this study was, through a systematic review, to take stock of this system.

**Methods** An electronic search on the PubMed database attempted to identify all relevant studies regarding fast curing resin composites. Papers published in English were identified after a review of their titles, abstracts and full text. The inclusion criteria were all well-conducted studies on this system, i.e. the Adhese Universal (Ivoclar Vivadent) adhesive, the Tetric PowerFill (Ivoclar Vivadent) sculptable bulk-fill resin composite or the Tetric PowerFlow (Ivoclar Vivadent) flowable bulk-fill resin composite, light-cured with the Bluephase PowerCure (Ivoclar Vivadent) high-power curing light.

**Results** Short light exposure time with high radiant exitance seems to have no adverse (or almost) consequences on: degree of conversion, depth of cure, flexural modulus, Vickers microhardness, polymerization shrinkage and shrinkage stress. The bulk-fill resin composites also show less or no internal defects. However, the shrinkage force kinetics is affected and the clinical tolerance for 3s irradiance should be limited to an exposure distance of 5mm, by avoiding angulation.

**Conclusions** Taking into account the latter precautions, fast-curing resin composites seem to be relevant and reliable for clinical use.
Shrinkage Vectors in MOD-Cavities With Different Applications of Bulk-Fill Composites
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Objectives
Optimally restoring cervical margins of class II direct resin composites is a particular clinical challenge. This study investigated different application methods of low and high-viscosity bulk-fill composites on the polymerization shrinkage vectors in mod-cavities with relevance to cervical margins.

Methods
MOD-cavities were prepared in 54 third molars with one box 6mm and the other 4mm deep, bonded with a self-etch adhesive (Adhese: groups 1-3; Scotchbond Universal: groups 4-6). In group-1, flowable bulk-fill composite Tetric_PowerFlow (PFL) was applied as flowable liner (2mm) in the deep box followed by bulk-filling the whole cavity with hybrid bulk-fill composite Tetric_PowerFill (PFI). In group-2, deep box was filled (2mm, PFL), proximal walls were built (PFI) and occlusal cavity was filled (PFI). In group-3, flowable bulk-fill composite PFL was applied in both boxes and the occlusal cavity then covered with hybrid bulk-fill composite PFI (2mm). Groups 4-6 were repeated with another flowable and hybrid bulk-fill composite: Filtek_Bulk_Fill_Flowable (FF) and Filtek_One (FO). Each composite increment was scanned in the micro-CT (uncured, cured) at standard resolution (16µm). Scans were subjected to data processing for shrinkage vector evaluation. Mean values were evaluated (one-way ANOVA, post-hoc Tamhane’s T2 test (p<0.05)). SEM images investigated the tooth-restoration interface.

Results
Largest vectors were observed in gp-6-FF (34.8µm), followed by gp-4-FF+FO (30.9µm), smallest vectors in gp1-PFL (12.5µm) and gp2-PFL (13.1µm). Flowable composite shrank toward tooth structure and restoration centre, but on applying the hybrid composite, it moved slightly upward. In general, FF and FO had larger shrinkage vectors than PFL and PFI, but the sequence of values were similar in the corresponding applications. Larger vectors were related to larger volumes. Only minute areas of debonding were occasionally observed at internal cavity corners.

Conclusions
The vector length varies depending on composite composition and its application method, more specifically the volume of each respective application.

Table 1 Mean values (µm) of shrinkage vectors and their standard deviation

<table>
<thead>
<tr>
<th>Group</th>
<th>Group with successive increment(s)*</th>
<th>Shrinkage vectors (µm) ± standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1</td>
<td>Gp1-PFL</td>
<td>12.5 ± 5.1 (a)</td>
</tr>
<tr>
<td>Group1</td>
<td>Gp1-PFL+PFI</td>
<td>19.5 ± 13.6 (f)</td>
</tr>
<tr>
<td>Group2</td>
<td>Gp2-PFL</td>
<td>13.1 ± 5.6 (a)</td>
</tr>
<tr>
<td>Group2</td>
<td>Gp2-PFL+wl/PFI</td>
<td>16.7 ± 10.2 (d)</td>
</tr>
<tr>
<td>Group2</td>
<td>Gp2-PFL+wl/PFI+PFI</td>
<td>15.5 ± 12.8 (c)</td>
</tr>
<tr>
<td>Group3</td>
<td>Gp3-PFL</td>
<td>22.2 ± 11.8 (g, h)</td>
</tr>
<tr>
<td>Group3</td>
<td>Gp3-PFL+PFI</td>
<td>14.5 ± 9.8 (b)</td>
</tr>
<tr>
<td>Group4</td>
<td>Gp4-FF</td>
<td>17.6 ± 8.4 (e)</td>
</tr>
<tr>
<td>Group4</td>
<td>Gp4-FF+FO</td>
<td>30.9 ± 36.6 (k)</td>
</tr>
<tr>
<td>Group5</td>
<td>Gp5-FF</td>
<td>19.3 ± 10.8 (f)</td>
</tr>
<tr>
<td>Group5</td>
<td>Gp5-FF+wl/FO</td>
<td>17.4 ± 9.7 (e)</td>
</tr>
<tr>
<td>Group5</td>
<td>Gp5-FF+wl/FO+FO</td>
<td>23.3 ± 25.3 (h, j)</td>
</tr>
<tr>
<td>Group6</td>
<td>Gp6-FF</td>
<td>34.8 ± 28.6 (l)</td>
</tr>
<tr>
<td>Group6</td>
<td>Gp6-FF+FO</td>
<td>24.7 ± 27.8 (h, j)</td>
</tr>
</tbody>
</table>

* Abbreviations of group names: Tetric PowerFlow (PFL), Tetric PowerFill (PFI), Filtek Bulk Fill Flowable (FF), Filtek One (FO); different letters mark statistically significant differences between the groups or separate increments.
Data Processing Parameters Influence the Shrinkage Vector Evaluation of Composites
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1LMU Munich, München, Germany, 2Imam Abdul Rahman Bin Faisal University, Dammam, Saudi Arabia, 3Faculty of Applied Sciences, Augsburg, Germany

Objectives
The shrinkage vector evaluation is based on different steps of data processing: rigid registration followed by sphere segmentation and registration based on a block-matching algorithm which is regulated by several parameters.

Methods
MOD-cavities were prepared in 53 fourth molars with one box 6mm and the other 4mm deep, bonded with a self-etch adhesive (Adhese; Scotchbond_Universal). Four different bulk-fill composites were inserted into mod-cavities in different applications: Tetric_PowerFlow (PFL) and Tetric_PowerFill (PFI) by Ivoclar Vivadent in half the specimen and Filtek_Bulk_Fill_Flowable (FF) and Filtek_One (FO) by 3M in the other half of specimen. Each composite was mixed with 2wt% radiolucent glass beads to serve as tracers for the displacement movement upon polymerization. Each composite application was scanned in the micro-CT (Scanco_40) in the uncured and cured state at standard resolution (16µm). In FF and FO the central restoration part was lacking shrinkage vectors. Investigating and adjusting some parameters of the data processing could improve the shrinkage vector evaluation.

Results
The shrinkage vector evaluation was successful in PFL and PFI throughout the whole cavity. The data processing in FF and FO needed adjustments of two parameters. First, the sphere grey value range used for thresholding the spheres was set differently based on the grey values specific to these materials. Second, the sphericity test parameter was increased to allow less round particles or spheres to be traced. This was the case when two glass beads were lying in close proximity to each other (touching) and the software mistakenly omitted these spheres as they were not detected as perfect spheres.

Conclusions
Knowing the details of data processing and adjusting some parameters enabled segmentation and registration of spheres with imperfect sphericity and composites with different radiopacity.

Properties of Dental Composite With Bisguaiacol-Based Monomer Replacing BisGMA
Line Etiennot1, Xin Li1, Fei Zhang2, Laura Trullemanš3, Marko Turkalj2, Fatima Rammal3, Ben Mercelis1, Bert Sels3, Kirsten Van Landuyt1, Marleen Peumans1, Bart Van Meerbeek1
1Department of Oral Health Sciences, BIOMAT & University Hospitals Leuven (UZ Leuven), Dentistry, KU Leuven (University of Leuven), Leuven, Vlaams-Brabant, Belgium, 2Department of Materials Engineering, MTM, KU Leuven (University of Leuven), Leuven, Flemish Brabant, Belgium, 3Department of Microbial and Molecular Systems (M2S), Centre for Sustainable Catalysis and Engineering (CSCE), KU Leuven (University of Leuven), Heverlee, Flemish Brabant, Belgium

Objectives
Concerns have been raised about the elution of Bisphenol-A (BPA) from dental resin-based composite (RBC). Increasing public awareness calls for the development of new dental restorative materials with a higher degree of biocompatibility. KU Leuven research has recently synthetized a bio-based platform of safer bisguaiacols, hence potential drop-in precursors for the synthesis of dimethacrylate resins, similar to bisphenol A dimethacrylate (BisGMA). This study aimed to analyse the degree of conversion (DC), elastic modulus (EM), flexural strength (FS) and Knoop hardness (KH) of experimentally prepared composites containing either bisguaiacol P dimethacrylate (p,p'-BGPGMA) or BisGMA in combination with TEGDMA and loaded with barium borosilicate glass, as compared to Clearfil Majesty ES-2 Classic (Clearfil-M; Kuraray Noritake) serving as reference.

Methods
Twenty bar-shaped specimens per composite were mirror-polished on one side. DC was assessed with micro-Raman spectroscopy at time points uncured, 30 min, 2 hrs, 72 hrs and 1 week after light-curing. EM was determined using impulse excitation, upon which FS was measured using four-point bending. Fractographic analysis was carried out using SEM. KH was measured using a micro-hardness tester. Statistical analysis involved One-Way ANOVA and Tukey’s multiple comparisons test (p<0.05).

Results
The significantly highest 1-week DC was recorded for p,p'-BGPGMA and BisGMA, as compared to Clearfill-M. Also EM and FS of p,p'-BGPGMA and BisGMA significantly exceeded that of Clearfil-M. KH of p,p'-BGPGMA was significantly higher than that of BisGMA and Clearfill-M.

Conclusions
The experimental p,p'-BGPGMA and BisGMA composites revealed superior properties compared to the commercial Clearfil-M reference. The p,p'-BGPGMA monomer resulted in at least equal mechanical and physical properties as compared to BisGMA.
Surface Properties and Microhardness of Composites After Polishing and Aging
S. Gizem Ülkü, Nimet Ünlü
Selçuk University Faculty of Dentistry, Konya, Turkey

Objectives The purpose of this study was to evaluate the effect of different polishing systems and aging on the color stability, surface roughness, microhardness of different composites.

Methods Samples prepared from Filtek-Ultimate-Universal (FUU), Clearfil-Majesty-E5-2 (CME), Palfique-Estelite-Paste (PEP), G-aenial-Universal-Injectable (GUI), Tetric-PowerFill (TP) composites (n=10) were polished with polishing discs (OneGloss (OG), Twist-Dia (TD), Nova-Twist (NT), Zenit-Flex (ZF)). The values measured without surface treatments were accepted as the control group (C). After polishing, the measures were repeated. Specimens were immersed in coffee, thermocycled between 5-55°C for 10000 cycles, equal to 1 year, final measurements were recorded. SEM and AFM images were taken to examine the surface properties (n=1). Data were analyzed with 2-way ANOVA, Bonferroni and paired sample t test.

Results Color change was highest in FUU which polished with OG, TD, NT groups and in PEP which polished with ZF groups (p<0.05). When the surface roughness of each composite was compared in the 4 polishing systems, significant differences were observed in FUU, CME, TP which polished with OG (p<0.05). When the roughness changes of the polishing systems between the composites was compared, higher roughness change was found only in the CME polished with OG (p<0.05). Significant differences were detected between the 5 composites in each polishing system in terms of microhardness change (p<0.05). However, the change in microhardness values of PEP samples was the most significant in all polishing systems (p<0.05). When the microhardness change of each composite was evaluated according to 4 different polishing systems; significant difference was observed in CME, GUI, TP groups (p<0.05). SEM and AFM images were consistent with the results obtained.

Conclusions While differences were observed between composites in color stability, roughness and microhardness values after coffee immersion and aging, changes were determined according to the polishing system.

Influence of Probiotics on the Roughness, Microhardness, Sorption/Solubility of Composites
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Dentistry, Bezmialem Vakif University, Istanbul, Turkey

Objectives The aim was to investigate the surface roughness (Ra), microhardness (VHN), sorption/solubility values of various resin-based materials by immersing in a probiotics sachet.

Methods A total of 100 disc-shaped samples (5mm x 2mm) were prepared from a flowable-hybrid composite (Charisma Flow), a self-adhesive-flowable composite (Vertise Flow), a nanohybrid-universal composite (OptiShade), and two bulk-fill composites (SonicFill and 3M Filtek One Bulk-Fill). After the surfaces were polished, samples were divided into two subgroups (n=10) and immersed for 10 min/day for 1 month in either a probiotics sachet (Reflor) or distilled water (DW). Ra, VHN, and sorption/solubility values were measured before and after immersion. Collected data were analyzed using using one-way ANOVA and Duncan tests at p<0.05.

Results No statistically significant difference was observed in the Ra values of the tested materials when immersed in DW or probiotic sach (p>0.05). However, probiotics sachet formed rougher surfaces than DW among samples. 3M Filtek bulk-fill composite showed the highest VHN after immersion in DW (53.96±2.52), and Charisma Flow showed the lowest VHN values when immersed in DW (24.61±2.66). The sorption and solubility values for the Vertise Flow was found to be significantly higher than the other tested materials (14.26±3.04, 6.36±1.19, respectively). 3M Filtek bulk-fill composite showed the lowest solubility value when immersed in DW (0.63±1.42).

Conclusions Various restorative materials with different structures tested in the present study can be used in patients who use probiotics supplements.
Biomechanical Analysis of the Root Filled Tooth Restored by Reinforced Composite
Raphael Richert\textsuperscript{1}, Hamdi Jma\textsuperscript{2}, Philippine Simonis\textsuperscript{3}, Maxime Ducret\textsuperscript{4}, Cyril Villat\textsuperscript{1}, Naji Kharouf\textsuperscript{3}, Davide Mancino\textsuperscript{3
\textsuperscript{1}\textsuperscript{2}Restorative Dentistry & Endodontics, Lyon Dental Hospital, Lyon, France, \textsuperscript{3}Mechanics Department, University of Strasbourg, Strasbourg, France, \textsuperscript{4}Department of Endodontics and Conservative Dentistry, Faculty of Dental Medicine, Université de Strasbourg, Strasbourg, France, \textsuperscript{5}Université Lyon 1, Villeurbanne, None Selected, France

\textbf{Objectives} The aim of the present study was to investigate the mechanical behaviour and the fracture resistance of a new multi-fiber-reinforced composite (mFRC) post compared to a conventional single fiber-reinforced-composite post (sFRC).

\textbf{Methods} An intact human maxillary premolar, extracted for orthodontic reasons, was scanned by using a micro cone beam computed tomography with a slice thickness of 0.15 mm. The segmented 3D image was modified to model the three restorative strategies: no post, mFRC and sFRC for a class II mesio-occlusal (MO) cavity. The attributed material properties were referenced from the literature and an oblique load of 300 N was applied on the vestibular cuspid of the premolar to simulate masticatory forces. The finite element analysis was conducted on the software Abaqus (Dassault Systèmes, Vélizy-Villacoublay, France) to calculate the strain and von Mises stresses of the premolar. For experimental tests, eighteen premolars were treated endodontically and MO cavities were prepared. All posts were cemented using dual-cure resin cement and restored with direct composite. A 0.3-mm-thick silicone layer was applied to simulate the periodontal ligament. After thermomechanical ageing, all specimens were tested under a 45° oblique load, and the maximum load was recorded in Newton (N). Data were statistically analysed using one-way ANOVA test.

\textbf{Results} For all models, high stresses were present on the lateral parts of the root with low stresses in the center of the root. Higher cervical root stresses and resin strains were present for models of sFRC compared to models of mFRC or without post. The results showed that mFRC posts had significantly higher fracture resistance (442.157±36.4 N) than sFRC posts (207.3±30.9 N). (P<0.05).

\textbf{Conclusions} Multi fiber reinforced composite represents an innovative but very young technique to restore severely damaged premolars. According to this study, root canal should be prepared without extensive dentin removal for prosthetic purposes. Further in vitro investigations are now required to investigate if these differences in mechanical behaviour are also significant for different amounts of tissue such as occlusal or mesio-occluso-distal cavities.

Relation of Modern Resin-Based Hybrid Composites to Tooth Structure
Nicoleta Ilie
Department of Conservative Dentistry and Periodontology, University Hospital, LMU, Munich, Germany

\textbf{Objectives} The study aims to compare the way modern resin-based composites (RBC) respond to mechanical stress related to the tooth structure they are designed to replace.

\textbf{Methods} Eight representative light-cured RBCs, including ormocers, gimmers, RBCs with nano and agglomerated nanoparticles, pre-polymerized, or compact fillers, were selected. Flexural strength/FS and modulus/E were measured in a 3-point-bending test. A fractographic analysis determined the origin of fracture. The quasi-static (indentation hardness/HIT, indentation modulus/EIT) and viscoelastic (storage modulus/E', loss modulus/E'', loss factor/tan δ) behavior was assessed by a depth-sensing indentation test equipped with a DMA module. One and multiple-way analysis of variance (ANOVA), Tukey honestly significant difference (HSD) post-hoc tests (\(\alpha = 0.05\)), and Weibull statistics were applied.

\textbf{Results} Parameter material exhibited the highest effect on E (\(p < 0.001, \text{partial eta-squared } \eta^2 = 0.857\)), followed by FS (\(\eta^2 = 0.729\)), and the strain (\(\eta^2 = 0.553\)). Highest material reliability was identified in the RBCs with nano and agglomerated nanoparticles. The most frequent type of failure originated from volume (81.3%), followed by edge (10.6%), and corner (8.1%) flaws. Enamel evidenced three times higher H\(\text{IT}, \text{E}\), and E' values as RBCs and dentin, and the smallest deviation from ideal elasticity. Ormocers exhibited the highest damping capacity, followed by the RBCs with pre-polymerized fillers. Damping capacity and static mechanical properties are mutually exclusive.

\textbf{Conclusions} Analyzed RBCs and the tooth structure are better adapted to the relevant frequency for chewing than for higher frequencies. RBCs are comparable to dentin in terms of their mechanical performance, but apart from the damping behavior, they are far inferior to enamel.

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O129

O130

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Nicoleta Ilie
Department of Conservative Dentistry and Periodontology, University Hospital, LMU, Munich, Germany

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Oral Health in Drug Abusers: an Observational Study.

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Objectives Drug/alcohol abusers often neglect their own health. The aim of this study is to report about their oral status and the effect of motivation to oral hygiene procedures on their psychological condition.

Methods Specific Rehabilitation Communities were involved. Patients underwent a first recruitment with an informed consent and anamnestic questionnaire. Then patients accepting were checked for several parameters: DMFT, Winkel Tongue Coating Index (WTCI), organoleptic detection of halitosis (Yaegaki & Coil Score 2000), substance and time of abuse, oral effects of the substance. Subsequently, subjects are asked to have Periodontal Chart of Berna filling, professional scaling and motivation. People are checked 15 days later.

Results Patients initially recruited were 53. Only 43 accepted the first step. Among them:
32.6% (14) was women and 67.4% (29) was men.
Mean age 45 years.
53.5% (23) was HCV+.
4.7% (2) was HIV+.
51.2% (22) was smokers.
41.8% went the last time to the dentist at least 1 year before, while 30.23% (13) at least 10 years before.
48.9% (21) had DMFT ≥ 15.
79.1% (34) had WTCI ≥ 3.
48.8% (21) had halitosis score ≥ 3.
Regarding the abuse substances, 58.1% (25) was multi-users, 23.3% (10) was heroin addicted, 4.7% (2) was cocaine addicted and 13.9% (6) was alcoholic.
51.2% (22) had a story of abuse longer than 10 years. Most reported oral signs during the abuse period were xerostomia, teeth grinding and clenching, TMJ disorders, temporary maxillary anesthesia.
Only 28 patients attended the second step. Among them:
78.6% (22) had FMPS and FMBS ≥ 15%.
39.3% (11) had a mean PPD ≥ 4 mm.

Conclusions Oral condition becomes poorer over the years of abuse. Several patients rediscover self-esteem after oral health motivation. Prevention and timely treatment in this fragile class can make the rehabilitation easier.
O132
Translation and Cross-Cultural Adaptation Involving Multi-Ethnolinguistic Population
Prashanti Eachempati1,2, Sumanth Kumbargere1,2, Mona Nasser1, Sally Hanks1, John Martin1
1Peninsula Dental School, University of Plymouth, Plymouth, United Kingdom, 2Manipal University College Malaysia, Melaka, Malaysia

Objectives Although uncertainty has always been an inherent aspect of human existence, the Covid-19 pandemic has reinforced this reality more than ever. Intolerance of an individual towards uncertainty (IU) determines their cognitive, emotional, and behavioral responses. We intend to determine the IU in dental patients as a part of the wider study which would explore how individuals with different IU levels respond to uncertainties in oral health decisions.

Our aim was to translate, cross-culturally adapt and validate Bahasa Malaysia (BM) version of Intolerance of uncertainty- short form (IUS-12) and propose a unique toolkit when involving multi-ethnic, multi-linguistic population.

Methods Translation meant for multi-ethnic, multi-linguistic people involves a layer of complexity, as the target language may be first translated by the reader into their mother tongue before answering the questionnaire. One example is Malaysia, where three predominant ethnic groups (Malay, Indian, Chinese) speak a common national language (BM) while having their own mother tongue.

Forward and backward translations were done by tri-lingual translators of the three ethnic groups. Translations were completed by not only focusing on the source and target language but also on whether the item retains its meaning in their respective mother tongue.

Cross-cultural adaptation involved the three ethnic experts with an innovative ‘incremental disclosure’ method for reviewing the semantic, conceptual, idiomatic, and experiential equivalence.

Results Two translators each from the three ethnicities participated in the translation and equivalence testing. Our novel approach involving all ethnicities together provided opportunity to identify the discrepancies and similarities of terms across the languages and aided in the development of the 5I’s toolkit.

Conclusions Migration of skilled professionals to different geographical locations, especially high-income countries such as the United Kingdom, has resulted in multi-cultural societies. Health research in such diverse population requires linguistically adapted instruments not only to overcome the lack of English language proficiency, but also to initiate a step towards health equity. The 5I’s toolkit may be beneficial to future researchers conducting cross-cultural adaptation for such diverse populations.

O133
Association Between Anxiety and Oral Health-Related Quality of Life
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Objectives This study aimed to explore the factors associated with oral health-related quality of life in a population with high dental anxiety

Methods Data were obtained from patients attending a therapeutic treatment program for people who have experienced torture or abuse and those with dental phobia (TADA) in Norway (n = 107). Patients completed a questionnaire including the Index of Dental Anxiety and Fear measuring dental anxiety, the Anxiety subscale of the Hospital Anxiety and Depression Scale measuring general anxiety, and Oral Impacts on Daily Performance measuring oral health-related quality of life. The various measurement instruments were evaluated psychometrically, and the variables associated with oral health-related quality of life were explored using hierarchical multiple regression.

Results Symptoms of dental anxiety and general anxiety were high, while oral health-related quality of life was poor. Dental anxiety, increasing age, increasing number of years since the last dental treatment, and higher general anxiety were significantly associated with lower oral health-related quality of life. The strongest association was found between general anxiety and oral health-related quality of life (-0.41, p<0.01).

Conclusions In conclusion, the factors associated with oral health-related quality of life in a population with high dental anxiety revealed a complex picture of dental anxiety. Dental practitioners who treat patients with high dental anxiety should be aware of the complexity of dental anxiety and be prepared for how it may complicate the therapeutic setting.
Prevention and Intervention Measures With the Regard to Eating Disorder.

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Objectives The objective of the study was to identify selected variables determining the taking of prophylactic and preventive measures in people with suspected/diagnosed eating disorders during basic medical and dental care, including an analysis of the level of awareness, knowledge, motivation, and communication by dentists and primary care provider practicing in primary professional health care in an urbanized area with a high population density.

Methods In the Upper Silesia region (Katowice, Bytom, Zabrze, Zawiercie), 138 questionnaires prepared for 2 groups of doctors were directly conducted: 89 doctors working as part of the primary health care practice and 49 dentists working as part of the dental practice in 2018-2020. The following parts were prepared in the questionnaire for both medical groups: 1. Demographic. 2. Epidemiological and diagnostic. 3. Clinical and therapeutic.

Results The knowledge of the three main units of eating disorders and the age of the highest risk of developing the disease was sufficient to initiate appropriate medical intervention. The standardized SCOFF screening question scheme was known to physicians (77.1%), at least in terms of one basic question identifying patients with the above-mentioned disorders. Dentists included enamel and dentin erosion (75.5%) as well as calculus and caries (51.0%) among the most common symptoms of eating disorders in the oral cavity. However, regarding the selection of the concentration of fluoride-based preparations, the use of other remineralization agents and dental materials with adhesive systems, dentists have definitely not distinguished separate protocols of treatment.

Conclusions In both groups, doctors and dentists demonstrated the knowledge of the main symptoms of patients with eating disorders, which made it seem that they were sufficiently prepared to undertake medical and dental interventions. The observed relationships between the experience of doctors and dentists and the level of knowledge and medical intervention prove the role of postgraduate training, updating the level of knowledge and skills in medical professions. It seems that, similarly to doctors, the ability to combine professional work and continuous improvement of professional qualifications after obtaining a medical or medical dental diploma is of key importance.

The Impact of Bariatric Surgery on Oral Health: a Nationwide Longitudinal Register Based Study

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Objectives The aim was to investigate the impact of bariatric surgery on the risk for dental interventions.

Methods All adults who underwent bariatric surgery in Sweden between January 1st 2009 and December 31st 2018 were identified in the nationwide Scandinavian Obesity Surgery Registry (SOReg; n=53,643). A control cohort was created by Statistics Sweden, matched 10:1 on sex, age and place of residence (n=536,430). The control cohort was assigned pseudosurgery dates corresponding to the surgical patient they were matched on. All individuals were followed in the Swedish Dental Health Register regarding event rates for four types of dental intervention: restorative, endodontic and periodontal interventions, and tooth extractions.

Results The surgical cohort had increased intervention rates regarding direct restorations (IRR 1.3; 95% CI 1.2-1.3), tooth extractions (1.2; 1.2-1.3) and endodontic interventions (1.2; 1.2-1.3) in the postoperative period compared to the preoperative period. Dental interventions were more common in the surgical cohort both pre- and postoperatively, compared to the control cohort. However, the difference between the groups increased markedly in the postoperative period. The between-group comparison postoperatively showed increased event rates for restorations (IRR 1.9; 95% CI 1.8-1.9), extractions (2.3; 2.3-2.4) and endodontics (2.3; 2.3-2.4).

Conclusions Bariatric surgery candidates have worse oral health than the background population. Surgery has an additional negative effect on oral health. Bariatric surgical candidates would benefit from preoperative counselling regarding oral health in order to minimize this effect.
Can Brushing Be an Alternative Treatment for Obesity?
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Objectives This study aimed to investigate whether orthodontic patients with extrinsic eating, emotional eating or restrictive eating disorders have a change in weight, nutritional attitudes and behaviors of individuals with better hygienic habits.

Methods To investigate our hypothesis, the Beck Depression Inventory, Eating Attitudes Test (EAT-40), and The Dutch Eating Behavior Questionnaire (DEBQ) were applied to healthy volunteers with a body mass index (BMI) of 25 and above, who applied for fixed orthodontic treatment in our clinic. Thus, individuals with extrinsic eating, emotional eating, and restrictive eating disorders and their eating habits were determined with psychiatrist consultation. Beck depression test, EAT-40, and DEBQ questionnaires were applied again at the 3rd and 12th months of fixed orthodontic treatment in 30 individuals with eating disorders. A questionnaire including the method, duration, and frequency of tooth brushing was made and the patients’ hygienic habits and gingival health status were measured using plaque index, gingival bleeding index, and pocket probing depth. All clinical measurements were performed by the same investigator.

Results After the correlation analysis patients are hierarchically clustered and correlation values are visualized with a heatmap using R. Correlation between brushing frequency and BMI change is observed to be statistically significant.

Conclusions Brushing frequency during fixed orthodontic treatment offers a potential treatment for obesity for patients without any metabolic eating disorder.

Subgingival Dysbiosis and Respiratory Decline.
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Objectives To investigate whether there was an association between subgingival dysbiosis and reduced respiratory function.

Methods A group of dentate 58-72 year-old men in Northern Ireland had a comprehensive periodontal examination including subgingival plaque sampling. DNA was extracted from plaque samples and the V3 to V4 regions of the 16S rRNA gene were analysed by high throughput sequencing and a microbial dysbiosis index (MDI) applied. Parallel to the periodontal examination, participants completed questionnaires gathering information on their medical history, social circumstances, demographic background and tobacco use. A physical examination assessed anthropometric measures. Spirometry measures were performed using a wedge bellows spirometer (Vitalograph S Model). The primary outcome variable of interest was the percentage predicted forced expiratory volume in one second (% predicted FEV1). Analysis included multiple linear regression with adjustment for various confounders.

Results 507 men were included in the analysis. The mean age was 63.6 years (SD 3.1). Applying the Page and Eke (2007) case definitions of periodontitis: 304 (60%) of the men had no mild/periodontitis; 105 (20.7%) had moderate periodontitis; and 98 (19.3%) had severe periodontitis. In terms of respiratory function, 108 (21.3%) men had a % predicted FEV1 < 80%. Multiple linear regression analysis showed that a 1 unit increase in the MDI was associated with a -0.68% loss (95% confidence interval -0.08% to -1.28%) p=0.03, in % predicted FEV1 after adjustment for all other predictor variables.

Conclusions In this homogenous group of dentate men, subgingival microbial dysbiosis, measured using a MDI, was significantly associated with a reduced respiratory function as measured by % predicted FEV1. Subgingival microbial dysbiosis may be a risk indicator for respiratory decline.
5-Year Tooth-Wear Progression in Severe Tooth-Wear Patients Using 3D-Measurements

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Objectives This study investigated the progression of wear over a period of five years in patients with generalized tooth wear using quantitative 3D wear-measurements.

Methods Fifty-five patients with near-complete dentitions and generalized tooth-wear without demand for restorative treatment were included in a prospective observational study. Intra-oral scans were made at baseline and after one, three, and five years using True Def Scanner (3M). Maximum height loss (µm) was measured per cusp/incisal/palatal surface on unrestored surfaces using the 3DWA-protocol with Geomagic Qualify (3D Systems, Germany), resulting in sixty-five maximum height loss measurements per dentition. A multilevel-regression analysis was performed.

Results Mean observation time was 2.8years±1.5 (N=46). Nine patients dropped out after intake. There were large inter-patient differences, varying between rates of wear of 29.0µm/y±20.7 and 265.4µm/y±197.4 (N=5809). Upper premolars showed highest wear progression rate (average 75.3µm/y±89.2) and lower premolars lowest (average 56.9µm/y±67.4). Functional cusps had higher wear progression rates (75.4µm/y±88.6 µm/y) than non-functional (62.6µm/y±81.1). The results of the multilevel-regression analysis are described in Table 1, reference is a non-functional upper premolar cusp.

Conclusions The method is precise enough to measure small differences in wear progression. There are multiple significant effects and interactions between jaw, tooth, and cusp type on tooth-wear progression. Large differences in wear progression were found between patients, confirming the need for individual management.

Table 1

<table>
<thead>
<tr>
<th>INTERACTION</th>
<th>EFFECT (µm)</th>
<th>95%-CI</th>
<th>P-VALUE</th>
</tr>
</thead>
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<td>69.1</td>
<td>[59.4 ... 78.8]</td>
<td>&lt;0.001</td>
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<tr>
<td>LOWER VS UPPER</td>
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<td>[-35.7 ... -20.3]</td>
<td>&lt;0.001</td>
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<td>0.02</td>
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<td>[-2.7 ... 8.4]</td>
<td>0.32</td>
</tr>
<tr>
<td>LOWER AND MOLAR</td>
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<td>[25.3 ... 46.6]</td>
<td>&lt;0.001</td>
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<tr>
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<td>[19.6 ... 35.4]</td>
<td>&lt;0.001</td>
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<td>[-5.9 ... 8.7]</td>
<td>0.71</td>
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<td>[-32.2 ... -11.6]</td>
<td>&lt;0.001</td>
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</table>
O140
Subsurface Characteristics of Erosive Tooth Wear on Natural Human Enamel
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Objectives This study explored why the outer layer of human enamel demonstrates a higher resistance to erosion, but when proceeded by abrasion, the wear is more comparable to polished enamel samples.

Methods Human unpolished (n = 20) and polished (n = 20) buccal enamel samples were embedded in acrylic resin and exposed to dietary acid (0.3% citric acid) for 20 minutes. Ten samples from each group were subsequently abraded via manual toothbrushing machine using unfluoridated toothpaste slurry mixed 3:1 with artificial saliva. Enamel loss was quantified with a non-contacting laser profilometer to produce high-density topographical scans. The digital profiles were superimposed and subtracted to calculate mean step heights. Six randomly selected unpolished samples were then sectioned through the lesion, and polished for SEM imaging.

Results The mean (SD) step height from the unpolished enamel after acid erosion was 1.52 (0.22) μm, and increased to 3.62 (0.39) μm after abrasion. The mean step height from polished enamel was 3.08 (0.4) μm and 4.08 (0.37) μm, respectively. The differences in mean step height between the unpolished and polished enamel samples following erosion, and erosion + abrasion were significant (p < 0.0001, p = 0.016 respectively); however the latter treatment yielded a smaller difference between the two enamel substrates. SEM images of each group revealed a distinct layer on the outer edge of the unpolished enamel where the enamel prisms had been exposed by the erosive challenge. HCL acid etching revealed a subsurface layer of altered prisms in the erosion-only group, which was not as clearly visible in the erosion + abrasion group.

Conclusions The outermost layer of enamel on the unpolished samples appeared to be more resistant to erosion from citric acid, but this resistance is reduced after combined chemo-mechanical attack. This study demonstrates the impact of mechanical abrasion on enamel surfaces that have been exposed to dietary acids highlighting the importance of the outer layer of enamel.

O141
Remineralisation and Protection of Human Enamel by Fluoride-Containing Dentifrice
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Objectives To quantify and visualise the ability of two fluoride-containing commercial dentifrices to remineralise and protect human enamel in-vitro by measuring the rehardening of erosive lesions, fluoride uptake, mineral density changes, and lesion formation visualisation.

Methods 60 human enamel samples were divided into three treatment groups (n=10): Sensodyne-Pronamel (SP), Crest Densify (CD), and Fluoride-Free Control (FF). Samples were immersed in citric acid, pH 3.8 for 10 minutes to create a representative erosive lesion. They were then incubated in a dentifrice slurry for 2 minutes, water-washed, and immersed in artificial saliva. SMH was taken at 24 and 48 hrs. Further 30 enamel samples were divided into two experimental groups (n=15) which were divided into three subgroups (n=5): SP, CD, and FF. Mineral density analysis was performed on the enamel following a 2-minute dentifrice slurry treatment and subsequent immersion in citric acid, pH 3.8 for 5 minutes using backscattered electron SEM (BSE-SEM). This treatment was reversed for the Fluoride uptake study using nano-scale secondary ion mass spectrometry (NanoSIMS).

Results SP was statistically superior at re-hardening erosive lesions. There were no significant changes to the enamel hardness following the second acid attack which demonstrates the ability of SP formulation to protect the dental tissue. Mineral density depth distribution also showed significantly higher resistance to erosive lesion formation after SP-treatment. Mean lesion depths after citric acid immersion were significantly lower for SP compared to the fluoride-free control. NanoSIMS revealed significantly higher fluoride uptake in the upper ~30μm of the enamel surface for SP-treated specimens.

Conclusions Dissolution of enamel minerals caused by acid attack leads to enamel erosion which can expose the underlying dentine and subsequently leave the dental tissue susceptible to caries and dentine hypersensitivity. This study has shown that SP can reharden the enamel and protect it against further acid attacks.
Characterization of Non-Carious Cervical Lesions

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Objectives Over the last century, modern societies have experienced a dramatic increase in the prevalence of dental pathologies. Non-Carious Cervical Lesion is one of those "modern" pathologies (NCCL). NCCL is defined as the non-bacterial loss of cervical tooth materials. NCCLs are prevalent worldwide and are commonly described as wedge-shaped or saucer-shaped. Nonetheless, the relationship between the shape of the lesion and its etiology remains unresolved. The purpose of this study is to characterize the different shapes and surface textures of NCCLs.

Methods Human teeth presenting eleven NCCLs were scanned using a microfocus X-ray computed tomography system (XT H 225 ST, Nikon Metrology NV, Leuven, the Kingdom of Belgium). The volume and surface area of the lesions were characterized using Amira software. The surface texture of the lesion was analyzed using a high-resolution confocal disc-scanning measuring system (Nanofocus AG, Germany).

Results The results indicated that the relative surface area/relative volume ratio was greater in the saucer-shaped compared to the wedged-shaped lesions (p=0.003). Additionally, the relative volume is greater in the wedge-shaped lesion (p=0.012). The surface texture pattern differed between various sites of the wedge-shaped lesion presenting a less homogenous pattern compared to the saucer-shaped.

Conclusions The wedge-shaped lesions have a larger volume with a relatively smaller surface area, whereas the saucer-shaped lesion shows the opposite pattern. The importance of revealing the morphological pattern of NCCLs has clinical significance as the wedge-shaped lesions spread toward the pulp, thus causing structural damage, hypersensitivity, and pulp exposure. Whereas the saucer-shaped lesions spread superficially and are less harmful to tooth strength. The homogeneous surface texture of the saucer-shaped lesion suggests the effect of a single etiologic factor acting uniformly on the cervical region. In contrast, the diverse surface texture of wedge-shaped lesions may indicate different etiological factors acting on the cervical area.
**Comparative Study of Toothpastes for Dentin Tubule Occlusion**

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**Objectives** The aim of this study is to evaluate the occluding/remineralization performance of herbal toothpastes.

**Methods** Eighty dentin samples were immersed in 6% citric acid solution for 2 minutes to expose the dentin tubules and remove smear layer. Dentin samples were randomly divided into 8 groups (n=10). Control (deionized water), (G) Gumgumix (herbal toothpaste with ginger-honey); Beka Ilaç, Turkey, (H) Experimental toothpaste with hemp (SPC Kozmetik, Turkey), (HO) Hemp oil, (S) Sensodyne Repair and Protect; GSK, Ireland, (C) Colgate Sensitive Pro-Relief; Colgate-Palmolive, Poland, (O) Oral-B Sensitivity and Gum Calm; P&G, Germany, (R) R.O.C.S. Sensitive, Repair and Whitening; R.O.C.S., Russia; agents were applied to the dentin surfaces using an electric toothbrush (Oral B, Cross Action, Procter&Gamble) for 1 minute 3 times a day for 1 week using the erosive cycle model.

Dentin samples were evaluated at baseline, after citric acid exposure and after treatment stages using Microhardness, FluoreCam, DIAGNOdent Pen, Ultrasound, Optical Profilometer methods and also the specimens were characterized with Scanning Electron Microscope. Data were statistically analyzed using ANOVA, Kruskal Wallis H, and Friedman tests (p<0.05).

**Results** According to FluoreCam data, lesion size decreased in herbal and conventional toothpastes and increased in hemp oil (HO) group, meanwhile all groups demonstrated mineral intake. Herbal toothpastes showed similar microhardness results to conventional toothpastes. The lowest microhardness values were obtained from HO (49.3 ± 3.65) and the highest microhardness value was recorded in S (76.6 ± 2.88) following C (74.9 ± 3.03); G (73.6 ± 3.03) and R (71.8 ± 2.9). According to DIAGNOdent Pen and Ultrasound results, all treatment groups except HO showed similar results which were significantly better than the other groups (p<0.05).

**Conclusions** According to the results of our study, our hypothesis has been confirmed that herbal toothpastes resulted in effective dentin tubule occlusion that may be used as an effective treatment to reduce dentin hypersensitivity.
Metabolic Disorders and the Risk of Head and Neck Cancer
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Objectives To systematically review and meta-analyse comprehensively the effect of a range of metabolic disorders and the risk of incident head and neck squamous cell carcinoma (HNSCC) across populations. To pilot the ROBINS-E (Risk Of Bias In Non-Randomised Studies - Of Exposures) to assess the risk of bias in the included observational studies.

Methods A search strategy was developed with an information and content specialists. Multiple databases including Cochrane Library, OVID SP versions of Medline, EMBASE and the grey literature were searched. The primary outcome for included studies was incident HNSCC and exposures of obesity, type 2 diabetes, dyslipidaemia, and hypertension, using World Health Organisation definitions. A combined risk effect across studies was calculated using a random-effects meta-analysis. Heterogeneity was assessed between studies using the Cochran’s Q and I² statistical tests. The ROBINS-E preliminary tool was used to assess the bias in each included result.

Results The search generated 7,316 abstracts, of these 197 full text articles were assessed for eligibility and 38 were included for full synthesis. Included were 10 studies reporting the association between BMI (n= 22,940 cases), 15 reporting type 2 diabetes (n= 17,582 cases), 6 studies reporting hypertension (n= 12,151 cases), and 4 studies reporting dyslipidaemia (n= 6,410 cases) and HNSCC risk. For combined head and neck cancer sites using random-effects models for each trait the following results were obtained; obesity RR of 1.00 (95%CI: 0.93-1.09, I²= 83.3%), type 2 diabetes mellitus RR of 1.13 (95%CI: 0.95-1.34, I² = 80.0%), hypertension (RR 1.10; 95%CI: 1.04-1.15, I² = 25.9%) and low-density HDL (RR 1.12; 95%CI: 1.07-1.18, I² = 62.5%).

Conclusions There is limited evidence of an effect of hypertension and dyslipidaemia on incident HNSCC, however caution is required due to the high levels of heterogeneity recorded.

Limited Effect of Genetically-Predicted Metabolic Disorders on Oral/Oropharyngeal Cancer Risk
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Objectives Obesity and related metabolic disorders such as type 2 diabetes (T2D) and hypertension have all been associated with head and neck cancer risk in multiple observational studies. However, selection bias, confounding or measurement error of both exposures and outcomes may explain the findings from these studies.

Methods To overcome the challenges of observational studies, we conducted two-sample Mendelian randomization (inverse variance weighted (IVW) method) using genetic variants which were robustly associated with obesity, T2D and hypertension in genome-wide association studies (GWAS). Outcome data was taken from the largest available GWAS of 6,034 oral/oropharyngeal cases and 6,585 controls.

Results We found limited evidence of a causal effect of genetically proxied body mass index (OR IVW 0.89, 95%CI 0.72–1.09, p = 0.26 per 1 SD in BMI (4.81 kg/m²)) on combined oral/oropharyngeal cancer risk. Similarly, there was limited evidence for genetically-proxied T2D (OR IVW 0.92, 95%CI 0.84–1.01, p = 0.09 per 1-log unit higher odds of T2D), or related traits including fasting glucose and insulin. Higher HbA1c resulted in a weak protective effect on combined oral/oropharyngeal cancer risk (OR IVW 0.56, 95%CI 0.32–1.00, p = 0.05 per 1-log-unit % higher HbA1c), which remained only in the oral subsite (OR IVW 0.48, 95%CI 0.24–0.93, p = 0.03) following stratification. Finally, there was limited evidence for an effect of systolic blood pressure on combined oral/oropharyngeal cancer risk (OR IVW 1.00, 95%CI 0.97–1.03, p = 0.89 per 1 unit mmHg increase).

Conclusions This analysis suggests the possibility that the effect of metabolic disorders may have been previously overestimated in observational studies. However, these cannot be directly compared given the differences in methodologies and the interpretation of estimates.

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Objectives
It is obviously known that anorexia nervosa (AN) is still one of the most prevalent eating disorders (ED) worldwide with uncertain prognosis. It is also noteworthy that adolescent malnutrition should be encouraged to undertake medical care, but whether young AN patients should be included to intensive oral care may be still questionable. In contrary, few scientific reports focused on oral status among AN individuals under age of 18y. affected by disease less than five years, i.e., during the first ED acute stage. This case-control study aimed to establish oral status regarding caries incidence, tooth wear, gingival inflammation and oral hygiene levels among severely ill adolescent inpatients diagnosed with AN.

Methods
Based on clinically confirmed 120 AN restrictive subtype hospitalized cases (BMI <15 kg/m², age 14.8±1.8), dental status has been examined regarding the occurrence of caries lesions using Decay Missing Filling Teeth (DMFT), erosive wear as Basic Erosive Wear Examination (BEWE), gingival condition as Bleeding on Probing (BOP), plaque deposition as Plaque Control Record (PCR) and compared with age-sex-matched 110 controls (BMI 19.8±2.3 kg/m², age 15.5±1.8, p=0.744) treated in public University dental clinic.

Results
AN patients compared with healthy adolescents were found to present higher incidence of oral-related complications according to dental status (DMFT 3.9±4.5 vs. 2.0±1.8, p=0.005), erosive tooth wear (BEWE 18.9% vs. 2.9%, p<0.001), controlling plaque (PCR 43.8% vs. 13.7%, p<0.001), gingival inflammation (BOP 20.0% vs. 3.9%, p<0.001). AN group, significant correlation between BOP, BEWE, and duration of AN symptoms (p<0.05), similarly to number of decayed teeth D, filled teeth F and PCR were detected (p<0.05).

Conclusions
Although obtained results did not reveal any severe oral complications, determination of AN restrictive subtype in adolescence may suggest numerous oral-related symptoms from dental caries, the onset of erosive tooth wear, gingival inflammation and failure to cope with dental hygiene. There is a need for accurate oral hygiene/diet instructions combined with regular oral check up visits to prevent forward oral disease progress. Therefore, each oral health assessment among young AN patients may provide important informations for dental care.

Image 1. Oral effects and care recommendations according to eating disorder symptoms
O150
Is Apical Periodontitis a Risk-Factor for Prevalent or Incident Coronary-Heart-Disease?
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Objectives To investigate apical periodontitis (AP) as a risk factor for prevalent and incident coronary heart disease (CHD) events in a group of middle-aged men from Northern Ireland.

Methods A representative sample of 1362 dentate men, aged between 58 and 72 years, had a comprehensive dental examination between 2001 and 2003. CHD events were validated by independent cardiologists. Periapical status was assessed from dental orthopantomograms using the periapical index. Men with AP were categorised as high (≥3 teeth with AP) or low (<3 teeth with AP). A cross sectional analysis was performed at baseline, the time of their dental examination, to explore associations between AP and prevalent CHD in men who were CHD-free at the beginning of the study and were followed-up until 2015 to investigate a potential association between AP and incident CHD. The chi square test was used.

Results The mean age of the men at baseline was 64.2 (SD 2.9) years. Of the 1362 men examined, 121 (8.9%) had prevalent CHD. The men who had prevalent CHD had fewer teeth (p=0.01), were less likely to have ever smoked (p<0.01) and had higher periodontal attachment loss (p<0.01), compared to those who had not experienced a CHD event. In the high AP group 38 (10.5%) of 363 men had a prevalent CHD event compared to 83 (8.3%) of the 999 in the low AP group, p=0.22. During a median follow-up of 12.7 (IQR 10.6-13.3) years, 134 (10.8%) of the 1241 men free of CHD at baseline had an incident event: 35 (10.8%) of 325 men in the high AP group had a CHD event compared with 99 (10.8%) of 916 men in the low AP group, p=0.99.

Conclusions In this group of dentate men, AP was not associated with prevalent or incident CHD.

O151
Poor Oral Health in People With Severe Mental Illness
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Objectives People with severe mental illness (pwSMI) were reported to have worse oral health than people without SMI, but there is still lack of evidence from large studies. This study aims to use big data approach to investigate the oral health status in pwSMI.

Methods Data from National Health and Nutrition Examination Surveys (NHANES1999-2016) and UK Biobank (UKB) were used. NHANES contains oral health measurements such as self-rated oral health, ache in mouth, tooth loss, periodontitis stage, and the number of decayed missing and filled teeth(DMFT); UKB has periodontal disease proxy such as painful/bleeding gum and loose teeth. PwSMI were identified using self-reported antipsychotic medication. Descriptive statistics were performed and comparison between pwSMI and people without SMI were made on their oral health measurements using t test or Chi-square test depending on the data type.

Results NHANES and UKB showed similar prevalence of SMI (718 out of 53348(1.3%) as pwSMI in NHANES; 6494 out of 502,505(1.3%) as pwSMI in UKB). NHANES showed that PwSMI were more likely to self-report poor oral health (23.1% vs 12.2%, p<0.001), more often to have ache in mouth (17.6% vs 7.6%, p<0.001), more likely to be edentulous (14.5% vs 6.7%, p<0.001), and have more DMFT (median difference 0.5, p=0.001) than people without SMI. PwSMI were also less likely to use dental floss everyday (18.6% vs 31.7%, p<0.001). UKB further showed that pwSMI were more likely to have periodontal disease (20.4% vs15.6%).

Conclusions PwSMI are more likely to suffer from poor oral health. Oral care for pwSMI should be a priority for care providers, and improving oral hygiene would lower their risk of oral disease and improve quality of life. Our study offered further evidence on the oral health status of pwSMI and emphasized the importance of oral hygiene for pwSMI.
O153

Infective Endocarditis – the Result of Dental Procedures or Daily Bacteraemia?

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Objectives A few years after the publication of the British guidelines, national recommendations were published by the Swedish Medical Products Agency in October 2012 promoting the cessation of antibiotic prophylaxis in dentistry for the prevention of infective endocarditis (IE). The aim of this study was to assess the association between oral streptococcal IE and invasive dental treatment among high-risk individuals.

Methods This nested case control study was based on a nationwide cohort study of 76,762 adult individuals (>17 years) living in Sweden from January 2008 to January 2018, with a diagnose code or surgical procedure code indicating high risk of IE. Definite and possible cases of IE caused by oral streptococci were identified using the national Swedish quality register for endocarditis. Controls (1:10) were sampled from the cohort and matched on age, sex, and risk factor. Occurrence of invasive dental procedures (tooth extraction, dental scaling, or oral surgery) in the 3-month period preceding diagnosis of IE was identified using the Swedish national dental health register, and compared using conditional logistic regression.

Results From 2008 to 2018, a total of 240 cases of oral streptococcal IE were identified among the 76,762 high-risk individuals in the original cohort. In 11 (4.6%) of cases and 80 (3.4%) controls, an invasive dental procedure was carried out in the preceding 3-month period. Four cases occurred after October 2012. Cases were not more likely to have undergone dental procedures than controls OR= 1.38 [95% Confidence Interval (CI) 0.72 to 2.66].

Conclusions The results of this study add to the growing evidence base suggesting that adherence to guidelines recommending antibiotic prophylaxis may do little to decrease the number of risk-individuals who develop IE. Oral streptococcal IE among high-risk individuals is more commonly associated with daily bacteraemia than invasive dental treatment.
Mediterranean Diet Increases Bone-Protecting Lipid-Mediators and Alveolar Bone-Micro-Architecture in Periodontitis

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**Objectives** Recent investigations described the inflammatory host response as the driving force in bacteria-induced periodontal disease and associated tissue damage. Therefore, concepts for novel therapeutic strategies concentrate on host-modulating risk factors and reducing a hyperinflammatory host response to prevent bone and soft tissue loss. Previous research has shown that obesity, especially in combination with a western-style diet enriched with palmitic acid (PA), increases inflammation and periodontal tissue breakdown in periodontitis. In contrast, intake of Mediterranean-style diets (MD) enriched with oleic acid (OA) results in a more anti-inflammatory and bone-protective condition. In the present study, we examined the inflammatory potential of these fatty acids in normal-weight-animal models. We focused on systemic and local activation of inflammatory signaling cascades and changes in oral bone micro-architecture associated with nutrition and infection.

**Methods** C57BL/6 mice were put for 16 weeks on isocaloric MD or WD enriched with OA or PA, respectively, and compared to normal-standard-diet (ND). Porphyromonas gingivalis (P. gingivalis) was used for periodontal infection. Jawbones were examined for cellular and histomorphometric changes using µCT and osteoclastic (OC) TRAP-staining. Serum analysis of inflammation markers using ELISA and lipid mediators (LM) using UPLC-MS-MS were performed. Effects of serum, cell culture supernatants and LM on bone homeostasis were investigated in vitro.

**Results** All animals were of normal weight irrespective of diet. Obesity-accompanying destructive effects of WD were largely absent. However, intake of MD protected oral bone-microarchitecture following P. gingivalis-infection and enhanced circulating pro-resolving mediators resolvin D4 (RvD4) and 4-hydroxydocosahexaenoic acid (4-HDHA). Cell culture experiments demonstrated significantly improved differentiation in RvD4- and 4-HDHA-treated primary osteoblast-cultures and reduced expression of osteoclastogenic factors in gingival fibroblasts.

**Conclusions** Our results demonstrate preventive impact of Mediterranean-style OA-enriched diets, indicating their pro-resolving nature beyond anti-inflammatory properties in periodontitis disease pathology.
**Guided Bone Regeneration in Osteoporosis by Plant-Derived Nanoparticles**

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**Objectives** The objective of our study was to promote bone regeneration using functionalised scaffold with Rhamnogalacturonan-I pectins (RG-I) in vitro and in vivo using aging and osteoporotic rodent models.

**Methods** The biomaterials were poly(l-lactide-co-ε-caprolactone) scaffolds. The chemical and physical properties were characterised with confocal and atomic force microscopy. Functionalised scaffolds with RG-I (tested sample) were evaluated in vitro with human osteoblasts from osteoporotic patients and their response was tested using real-time PCR. In vivo evaluation was performed using critical-size calvaria bone defect in aging and osteoporotic rat models. Scaffolds were implanted randomly in the calvaria defects of aged female Wistar rats (11-12 months old) and osteoporotic female Wistar rats induced by ovariectomy. The control was scaffold without RG-I. After 2 and 8 weeks animals were euthanised. Harvested samples were analysed for osteogenic and inflammatory markers using real-time PCR. Bone formation was evaluated radiographically and histologically.

**Results** The chemical and physical properties results indicated success of the functionalisation with RG-I. Osteoblasts response suggested osteogenic (upregulation osteopontin, osteocalcin, collagen1, bone sialoprotein) and anti-inflammatory properties (downregulation IL-1, IL-8, TNFalpha) on the scaffold with RG-I. The in vivo results in elderly and osteoporotic rat calvaria model of early (2 weeks) bone regeneration showed increase of osteogenic markers and decrease of proinflammatory markers and RANKL, compared to control. In osteoporotic rat model at week 2 and 8 and in elderly rat model at week 8, the mean percentage of bone volume/tissue volume in the defect with RG-I scaffold was significantly greater than the control. The histological evaluation in both rat models revealed larger areas of new bone formation in RG-I scaffolds than in control.

**Conclusions** In conclusion, the plant-derived nanoparticles significantly increased osteogenic and decreased pro-inflammatory response in vitro and in vivo. These finding may have a crucial influence on bone repair process especially in elderly and osteoporotic patients.
Liposomal Resolvin-D1 for Bone Remodeling: Based on Immunotherapy and Nanotechnology
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Objectives Local controlled bone growth stimulation is crucial in periodontal, orthodontic, and orthopedic treatments. Resolvin D1 (RvD1) is an endogenous lipid with a scientifically proved pivotal role in inflammation resolution. However, its potential in bone regeneration has been scarcely studied. Moreover, its clinical use is limited due to its short bioactivity. We aimed to elucidate the RvD1 potential anabolic effect on bone regeneration and develop a delivery system based on immunotherapy and nanotechnology, for RvD1’s local sustained release.

Methods RvD1 was loaded in florescent large liposomes (Lipo-RvD1). Encapsulation efficiency (EE), stability, and release were detected by ELISA, and in vivo bio-distribution by IVIS. Bioactivity test in MC3T3-E1 preosteoblasts that were cultured w/o RvD1 for 3 weeks. Osteoblasts’ markers were evaluated using qRT-PCR and Alizarin Red staining for calcified matrix. In vivo: Alveolar bone regeneration (ABR) model was performed via generating osseous defects in C57BLK mice. Defects were filled with allografts combined with (a) single free RvD1 (b) 4 repetitive injections of free RvD1(Rep-RvD1) (c) single lipo-RvD1 (n=7/group). 2w later, the sockets were analyzed using μCT, Masson-Trichrome & TRAP staining, and RNA sequencing.

Results In vitro, RvD1 encouraged osteoblasts differentiation and matrix mineralization. RvD1 was efficiently encapsulated in liposomes (>80%) and released 14.8±3.07% in 14d compared to free RvD1 that was washed away in 24h. In vivo: Liposomes resided for 3 days. Lipo-RvD1 increased bone content by 75±38% compared to Rep-RvD1 24±20%, and single RvD1 was effectless. Histologically, lipo-RvD1 reduced osteoclasts’ number and enhanced connectivity between allograft and new bone. mRNA sequencing analysis in Lipo-RvD1 vs controls revealed a total of 25 differentially expressed genes, associated with bone and tissue growth (Amelogenin, Col1a).

Conclusions We successfully developed a novel Lipo-RvD1 drug delivery system, which improved bone regeneration and osteoblast activation, suggesting it as a possible therapeutic drug product for bone regeneration.
O158
Implants-Derived Nanoparticles and Response of Gingival MSCs to Inflammatory Cytokines
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Objectives
Implant installation, corrosion, and therapeutic procedures like implantoplasty can cause the release of nano- and microparticles from dental implants. These particles are currently discussed as a potential risk factor for peri-implant disease. Resident gingival mesenchymal stromal cells (MSCs) play an essential role in soft tissue homeostasis, which is largely depending on their immunomodulatory function. In the present study, we compared the response of human gingival MSCs on titanium and zirconia nanoparticles in the presence and absence of various inflammatory cytokines.

Methods
Primary human gingival MSCs were isolated from six healthy individuals. Cells were treated with 25 or 100µg/ml of commercially available titanium and zirconia nanoparticles (<100 nm) in the presence or in the absence of interleukin (IL)-1β, tumor necrosis factor (TNF)-α or interferon (IFN)-γ for 24 h. Cell viability/proliferation was measured by the MTT method. Additionally, the gene and protein expression of pro-inflammatory IL-6, IL-8, and monocyte chemoattractant protein (MCP)-1, and immunomodulatory indoleamine-2,3-dioxygenase (IDO-1) and prostaglandin E2 (PGE2) were measured.

Results
Nanoparticles of both materials have not affected cell proliferation/viability. In the absence of inflammatory cytokines, both types of nanoparticles (>25 µg/ml) induced a significant expression of IL-8 in gingival MSCs. Slightly higher expression levels were observed for titanium compared to zirconia nanoparticles. Additionally, nanoparticles modified the response of gingival MSCs to various cytokine, which depends on the type of inflammatory stimuli and target protein. Under these inflammatory conditions, the expression of pro-inflammatory mediators was further enhanced by nano-particles, whereas the expression of immunomodulatory factor IDO-1 was inhibited by nanoparticles.

Conclusions
Nanoparticles influence the response of gingival MSCs to various inflammatory cytokines by altering the balance between inflammatory and immunoregulatory response of gingival MSCs toward inflammation. Thus, implant-derived nanoparticles could be considered as a risk factor in peri-implantitis.

O159
Immediate Restoration of Implants Using a Volume-Stable Collagen Matrix.
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Objectives
Connective tissue graft (CTG) in conjunction with immediate implant placement and restoration (IIPR) increases gingival thickness and diminishes recession and improves pink esthetics score (PES) compared to IIPR alone. Recently a volume-stable collagen matrix (VCMX) was introduced as a good alternative to CTG regarding the volume and stability of peri-implant soft tissue.

Methods
A case-control, prospective study involving 20 patients treated with maxillary anterior single implants, immediately placed and restored. All the patients presented compromised buccal bone treated by guided bone regeneration using native collagen membrane, xenograft, and VCMX. Implants were analyzed using standard clinical examination and a comprehensive index, comprising pink esthetic scores (PES). The height of the implant crown and the corresponding height of the contralateral tooth crown were measured to identify mucosal recessions. The distance from the mucosal margin to the implant shoulder (DIM) was measured on the master model at crown delivery. The volumetric soft and hard tissue changes between preoperative to immediately after crown installation (6 months) and 12-month follow-up were measured using the scanned models by a digital imaging software program.

Results
All 20 implants fulfilled the strict success criteria set up for dental implants regarding osseointegration. Mean PES of >6 (range between 1-10) defined as a good aesthetic result was achieved in 18 out of 20 implants. The highest mean values were achieved for the variable of root convexity/soft tissue color and texture.

Conclusions
The combined GBR and VCMX procedure achieved favorable peri-implant soft tissue conditions and esthetic results. However, recession and incomplete papillae were frequently observed.
New Radiographic Method to Measure Marginal Bone Level Around Implants
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Objectives Of all the existing definitions, implant success always agrees on the maintenance of the marginal bone level (MBL). The gold standard radiographic methodology to measure MBL was described by Weber and Buser in 1992. The aim of this preliminary study was to assess the accuracy of a new radiographic method to measure MBL and its concordance with the reference method.

Methods MBL was measured through radiographic assessment on digital peri-apical radiographs with a resolution exceeding 15 pixels/mm (>300 dpi) and examined with ImageJ software. The reference method was used for calibration and plotting. The test method proposed the use of gray levels profiles (GLP) for an unbiased computational determination of the first bone contact dense enough to represent the four bone walls. GLP were measured at distance from the MBL and radiolucent anatomic structures and at equidistant from any nearby implant, from mesial and distal aspects. The minimum GLP was selected as the reference to stop the MBL measurement. Lin’s Coefficient of Concordance ($CC_{Lin}$) was calculated to measure the repeatability (intraclass) and reproducibility (interclass) of the test method and to assess the concordance with the reference method.

Results For the test method, intraclass concordance $CC_{Lin}$ was of 0.97 and 1 for GLP and of 0.93 and 0.95 for MBL, for each examiner respectively (36 measures); and interclass concordance $CC_{Lin}$ was of 1 for GLP and of 0.81 for MBL between the two examiners (204 measures). For the MBL, between the reference and the test method $CC_{Lin}$ was of 0.46 (120 measures).

Conclusions GLP was a reliable and reproducible solution to overcome human reading errors concerning the distinction of gray levels whatever the x-ray reading conditions were. Regarding Buser’s method these preliminary results were not in concordance, without this outcome being able to determine which methodology comes closest to the real MBL.

Effect of Antibiotic Prophylaxis in Dental Implant Surgery - a Systematic Review
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Objectives The use of antibiotic prophylaxis in conjunction with dental implant surgery is a controversial topic. Despite no clear evidence, antibiotic prophylaxis is commonly administrated to patients in order to prevent an infection that can lead to implant failure. Given the undebated growth of antibiotic resistance, it is of great importance to reduce the use of antibiotics in dentistry and only use it in cases where the benefits are clear and greater than the risks. The aim of this study is to assess the effect of antibiotic prophylaxis in dental implant surgery regarding implant failure and postoperative infections.

Methods A search was performed in PubMed (Medline), Web of Science and The Cochrane Library, as well as a hand-search to find appropriate literature. Only primary studies were included and were assessed using GRADE.

Results Screening of abstract yielded fifteen studies warranting full text scrutiny. In total eleven articles were assessed for risk of bias. Quality assessment of the eleven studies disclosed five with high risk of bias and six with low risk of bias. None of the included articles with low risk of bias individually showed a statistical significant benefit of antibiotic prophylaxis with regards to implant failure and postoperative infections. At the moment an extraction of relevant data is being assessed and further statistical analysis of the appropriate data will be obtained.

Conclusions Results from this article could be used to lay the ground for new and clearer clinical guidelines concerning the use of antibiotic prophylaxis in conjunction with implant surgery. Preliminary conclusion is that antibiotic prophylaxis in conjunction with dental implant surgery is of small clinical relevance and should thus be avoided in most cases, especially considering the growth of antibiotic resistance.
**O162**

**Peri-Implantitis Effect on Dental Implants' Passivation Layer: From Biocompatible to Bio-Reject**

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**Objectives**

Dental implants are fabricated from Titanium and its alloys. These metals are considered biocompatible due to their passive oxide layer which promotes the osseointegration process. Peri-implantitis is a common disease caused by bacterial biofilm, in which the dental implant loses progressively its bone anchorage. The titanium oxide layer, which is in direct contact with the bacterial biofilm and inflammation process during disease progression, is probably affected by the environmental changes. This degradation, might modify the surface oxide properties, rendering it from bioinert to bio-reject.

**Methods**

The study systematically collected and document failed titanium dental implants due to peri-implant disease, which lost most of their bone support and were deemed hopeless. Next, a thorough evaluation of the implants’ surface was carried out using high resolution scanning methods such as XPS, FIB-SEM and TOF-SIMS on representative locations along the failed implant and the oxide layer nature (thickness) was determined together with a detailed characterization of the implant’s surface composition. The analysis included a comparison with reference (“out of the box”) unused implants with an intact native titanium oxide layer.

**Results**

The analysis has shown a clear change in the surface composition along the implant’s length. Areas which were still anchored to the bone have shown a surface composition like that of reference unused implant while in the used implant’s titanium oxide layer was found to be inconsistent in its width with large areas occupied by organic contaminations.

**Conclusions**

The study identified alterations of the titanium surface oxide layer properties, oxide layer thickness, continuity, impurity’s element content and amount, all of which can be tentatively correlated with the disease progression and possibly hinder the reestablishment of bone anchorage (re-osseointegration) even once the biofilm is removed and the inflammation ceased.

**O163**

**Shear-Bond Strength of Glass Ionomer-Based Materials to Calcium-Hydroxide Liner**

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**Objectives**

The aim of this study is to evaluate the shear bond strength (SBS) glass ionomer based materials to calcium hydroxide liner, with and without cavity conditioner application.

**Methods**

60 acrylic blocks with central holes (Depth:2 mm,diameter:5 mm) were prepared. The holes were filled with a calcium hydroxide liner (Dycal,Densply Caulk,USA) the specimens were allocated into 6 groups according to the cavity conditioner (GC Dental) application (CC application/No CC application) and the glass ionomer-based materials used [ A:glass ionomer cement (GIC) Fuji IX (GC Dental),B :Resin modified glass ionomer cement (RMGIC) Fuji II LC Capsule (GC Dental),C:bioactive ionic resin liner with reactive glass filler (BAL) Activa-Bioactive Liner (Pulpdent)] (n=10).

Group I:Dycal+GIC
Group II:Dycal+RMGIC
Group III:Dycal+BAL
Group IV:Dycal+Cavity Conditioner+GIC
Group V:Dycal+Cavity Conditioner+RMGIC
Group VI:Dycal+Cavity Conditioner+BAL

cylinder-shaped glass-ionomer based materials (diameter:2.4 mm,height:2 mm) were placed. The SBS test was subjected (1 mm/min) and failure type analysis were performed. Data were statistically analyzed with two way ANOVA and Bonferroni tests (p<0.05).

**Results**

RMGIC showed significantly higher SBS than GIC both with and without cavity conditioner (p<0.05). With and without cavity conditioner application, no significant differences in SBS were found between RMGIC and BAL groups (p>0.05). With or without conditioner, both GIC and BAL showed similar SBS (p>0.05). Applying conditioner did not cause significant differences in SBS of materials (p>0.05).

**Conclusions**

Regardless the conditioner application, resin modified glass ionomer exhibited higher shear bond strength than glass-ionomer cement. Bioactive Liner showed similar bond strength to resin modified glass ionomer cement.
Does Etching Mode Affect Bonding of Resin Composites to TheraCal-LC?

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Objectives This study aimed to compare the shear bond strength (SBS) of three different resin composites to pulp capping material TheraCal LC with different etching modes.

Methods Totally 180 acrylic blocks with central holes (2mm-depth, 4mm-diameter) were prepared and the holes were filled with TheraCal LC (BISCO Dental Products, USA). The specimens were randomly assigned to 3 groups (n=60) according to the resin composite used [conventional posterior composite-Estelite Posterior (EP)(Tokuyama, Japan), short-fiber reinforced composite-EverX Posterior (EX)(GC, Japan) and short-fiber reinforced flowable composite-EverX Flow (EF)(GC, Japan)]. Then, they were subdivided into 2 groups (n=30) according to the etching modes [self etch (SE) / etch and rinse (ER)]. Afterwards, a universal adhesive (Single Bond Universal, 3M ESPE, USA) was applied on TheraCal LC specimens and the resin composites were bonded. The bonded specimens were subjected to SBS test using a universal testing machine with a crosshead speed of 1 mm/min (AGS-1000D, Shimadzu, Japan) either immediately or after 10,000 thermal cycles (5-55°C) (n=15). Failure modes were examined with a stereomicroscope (25×) (SMZ 1000, Nikon, Japan). Data were analyzed using 3-way analysis of variance and Bonferroni test (p<0.05).

Results Regarding the immediate SBS values, the highest value was obtained in EF group in both etching strategies (SE: 18.89±7.53MPa, ER: 17.73±7.06MPa) (p<0.05) and there is no significant difference between the other groups (p>0.05). Only the SBS values of EF group decreased significantly after thermal cycling. There was no significant influence of the etching mode on the SBS values of tested materials (p>0.05).

Conclusions Short-fiber reinforced flowable composite did show the highest SBS values in both etching modes. Thermal cycling did affect SBS of short-fiber reinforced flowable composite to Theracal LC negatively. However, different etching modes used in this study did not affect SBS of tested resin composites to Theracal LC.

Is Marginal Seal in Class II Affected by Location/Placement Technique?

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Objectives Flowable restorative materials are less viscous than conventional composite resins. Using flowable composite could assist in restoring less accessible regions of the prepared cavity, it’s less controllable and might be more affected by gravity. Various techniques are offered for Class II restorations combining these materials. Aim: To assess the effect of restoration techniques, Pre-cured and Co-cured, and the location of the restored tooth in the oral cavity on the marginal seal.

Methods Pre-cured and Co-cured techniques using G-aenial Flo X and GRADIA DIRECT, were used to restore interproximal interfaces of extracted premolars. Plastic molds, 5 teeth in each mold, 3 in each quadrant were fitted into artificial jaws mounted in a phantom head model. The molds were filled with acrylic dental resin and polyether impression material resembling the bone and periodontal ligament, respectively. Flowable composite in the Pre-cured technique was cured before applying the conventional composite while in the Co-cured technique both composites were cured simultaneously. Mesial and distal sides were alternately restored by the two techniques. Aging was conducted by cyclic-loading and thermo-cycling. Teeth were examined after aging under SEM to determine the gap formed between the restoration and the tooth at the gingival wall.

Results A significant difference was found (p=0.016) in the mean gap between upper (4.516±1.782μm) and lower (3.796±0.879μm) jaws. No significant difference was found between the two techniques neither in mesial sides (p=0.462) nor in distal side (p=0.156).

Conclusions Performing composite restorations using both techniques in the upper jaw is a more sensitive than in the lower one when using flowable composite as a part of the procedure. This material is less viscous and more affected by gravity than conventional composite, therefore it’s less controlled by the practitioner.
Resin Matrix Composition Influences Bisphenol A Elution From Experimental Composites

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Objectives Only little is known about the kinetics of bisphenol A (BPA) release from composites. Therefore, experimental composites were prepared containing known amounts of BPA. The objective of this study was to determine which amount of the BPA initially present in the material leaches out over a seven-day period and how this release is influenced by the resin composition.

Methods BPA (0, 0.001, 0.01, or 0.1 wt%) was added to experimental composites containing 60 mol% BisGMA, BisEMA(3), or UDMA, respectively, as a base monomer and 40 mol% TEGDMA as a diluent monomer. For each material, polymerized samples (n=5) were immersed at 37°C for 7 days into 1 mL of water, which was refreshed daily. BPA release was quantified by UPLC-MS/MS after derivatization with pyridine-3-sulfonyl chloride.

Results The extent of BPA elution ranged between 0.34 to 0.65 mol%, depending on the resin composition and the added amount of BPA. BisEMA(3)-0.001 wt% released significantly more BPA, although no significant difference with BisGMA-0.001 wt% was observed after correction for the BPA content. BisGMA-composites containing 0.01 and 0.1 wt% released significantly more BPA. The release of BPA was significantly lower from UDMA-based composites, irrespectively of the amount of BPA added.

Conclusions Only a minor fraction of BPA between 0.34 and 0.65 mol% was released within one week. This study revealed that the resin matrix composition plays an important role in the release of BPA. BisGMA-composites released the highest amounts of BPA, presumably due to their limited polymerization, whereas UDMA-based composite may reduce BPA release.
O167

Influence of Denture Base Materials on the Attachment of Microorganisms
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Objectives The aim of the current study was to elucidate potential interactions between materials for the fabrication of denture bases and the attachment as well as proliferation of microorganisms, focusing on respiratory pathogenic microorganisms and Candida species.

Methods Specimens (6mm x 1mm) with a standardized surface roughness (Sa = 0.1 µm) were prepared from polymethyl methacrylate (PMMA), computer-aided-design/computer-aided-manufacturing (CAD/CAM) PMMA, and CAD/CAM polyether ether ketone (PEEK). Specimens were analyzed regarding their surface texture, chemical composition, and surface free energy. Subsequently, the specimens were randomly placed in the vestibular areas of professionally cleaned complete upper dentures in seven edentulous patients and removed either after 24 hours without any oral hygiene measures or after four weeks, in which the patients followed a standardized oral hygiene regime. Microorganisms adherent to the surface of the specimens were finally cultivated on seven different selective and non-selective nutrient media. Analysis of microbial attachment was performed by identifying all grown colonies using mass spectrometry (MALDI-TOF).

Results There was a general increase in total bacterial counts between the first (24 hours) and the second (four weeks) measurements. Regarding quantitative microbiological analyses, only minor differences were identified between the various materials. After four weeks, higher levels of Candida were detected. Respiratory microorganisms were identified on all specimens.

Conclusions Candida species and respiratory microorganisms accumulate on denture base resins.

O168

Residual Bioceramic Endodontic Sealer Evaluation in Endodontic Retreatment
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Objectives To evaluate the area of residual bioceramic endodontic sealer, after reshaping of root canals, previously filled with hydraulic condensation and bioceramic sealer BioRoot RCS (Septodont, France).

Methods Samples of 12 mm have been obtained by cutting off the coronal aspect of single rooted human extracted teeth (n=50). The root canals have been prepared and obturated with hydraulic condensation technique and bioceramic sealer with matching gutta-percha points (Pro Taper Gold). In group 1 only Pro Taper Retreatment (PTR) files are used. In group 2 ultrasonic tips (Pro Ultra 6, 7, 8) are used in conjunction with PTR files. In group 3 the Self Adjusting File (SAF) is used in conjunction with PTR files. In group 4 after PTR files, chloroform is added during the preparation with SAF for one minute, and in group 5 - 40% citric acid (Cercamed) is used instead of chloroform. After reshaping all the samples are split and subjected to digital optical microscopy (Flexion Advanced, CJ-Optics) and ImageJ® software is used to measure the area of residual bioceramic sealer for coronal, middle and apical third of the root canal. Data are statistically analyzed by ANOVA and Pairwise Comparison Analysis.

Results The total mean area of residual bioceramic sealer for group 1 is 0.45297 mm², for group 2 – 0.31021 mm², for group 3 - 0.35392 mm², for group 4 – 0.40441 mm², and for group 5 – 0.37074 mm². Significant statistical differences are found between group 1 and groups 2 and 4, group 5 and groups 2, 3 and 4 in the coronal third, and between group 2 and group 4 in the middle third of the root canal (p<0.05).

Conclusions BioRoot RCS cannot be completely removed from the root canal in the course of endodontic retreatment with the means used in this in vitro study.
Minimally Invasive Dental Implant: Topographical Surface and Biological Behaviour Assessment.
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Objectives
This study aimed to assess the topographical and the in vitro biological behavior of a minimally invasive implant compared to a gold standard implant.

Methods
Commercially minimally invasive implants MagiCore® (MC®, InnosBioSurg, IBS) and bone level NobelParallel™ (NB™, Nobel Biocare™) were investigated. Topographical characterization was performed using Scanning Electronic Microscopy (SEM), Energy-Dispersive X-ray spectroscopy (EDX), Atomic Force Microscopy (AFM), and profilometry. The biological behavior assessment was conducted regarding primary human gingival fibroblasts (hGF) and human osteoblasts like cells (MG63). Cell cytotoxicity and cell metabolic activity were evaluated using LIVE/DEAD™ staining and Alamar Blue™ assay respectively. SEM and confocal microscopy were used to evaluate cell adhesion and colonization. In addition, Alizarin Red staining and ALP activity were quantified to assess the mineralization potential of hGF cells. Data were analyzed by two-way ANOVA and Post Hoc tests.

Results
Roughness values for NB™ were Ra = 1.28 µm and Ra = 2.02 µm for MC®. Metabolic activity and cytotoxicity indicated equivalent biological development regarding both cell types for the two implants. Significant enhancement was found for hGF ALP activity in the presence of the two tested implants in a time-dependent manner from day 7 to day 14 (**p < 0.01). Alizarin Red S assay demonstrated significant enhancement of the extracellular calcium deposition when cells were interfaced with the NB™ compared to MC® implant (**p < 0.05). Further, both cell types exhibited good cell adhesion and spreading. Confocal imaging revealed a denser cellular layer on the MC® than the NB™ surfaces.

Conclusions
This study highlighted the minimally invasive implant ability to enhance oral cells adhesion and colonization despite the alternative topographical surface. These in vitro findings provide new insights about the biological behavior of the minimally invasive implant that could instigate its clinical use in different dental implantology situations.

Evaluation of Cytotoxicity of Toothpastes Used for Different Purposes
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Objectives
The aim of this in vitro study is to evaluate the cytotoxicity of toothpastes for different purposes.

Methods
Two daily toothpastes (Ipana Proexpert, Colgate Protection Maximum Anti-caries), three desensitizing toothpastes (Sensodyne Full Protection, Signal Sensitive Expert, Elmex sensitive) and two whitening toothpastes (Colgate optic white, Signal white now) were diluted (50 w/v%) (1:1, 1:2, 1:4, 1:8, 1:16, and 1:32) in medium. L929 fibroblast cells were treated with the medium containing the toothpaste extracts for 2 min. Cell viability was assessed using the methyl tetrazolium test. The viability of the negative control group was calculated as 100%, and the percentage viability of all groups was determined accordingly and statistically analyzed.

Results
The viability of cells exposed to 1:1 and 1:2 dilutions of all toothpastes were significantly reduced (p<0.05). At 1:4 dilution only Elmex Sensitive Expert was similar to the control group. At 1:8 dilution, Sensodyne Full Protection and Colgate Maximum Anti-caries and Elmex Sensitive Expert groups were similar to control (p>0.05). At 1:16 dilution, Signal White Now and Signal Sensitive Expert groups were different from control (p<0.05). At the 1:32 dilution, all groups were similar to the control (p>0.05).

Conclusions
According to the results of this study, toothpastes for different purposes may show cytotoxic effects. The most biocompatible results were seen in the Elmex Sensitive Expert group.
Learning Spaces in Undergraduate Dental Education: a Scoping Review Protocol
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Objectives
The objective is to develop an a priori protocol for a scoping review to explore the impact of learning spaces (LS) on undergraduate dental professional students.

Methods
Protocol development followed published guidance for scoping reviews, which is mapped against the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) 2015 statement, and aligns with the scoping review methodological guidance in the Joanna Briggs Institute Manual for Evidence Synthesis. The process included identification of the research questions, alignment of the objectives with the inclusion criteria, and confirmation of the approach to evidence selection, data extraction, analysis and presentation.

Results
The result of this process is a scoping review protocol, which has been designed to answer the following research question; what is the impact of LS on dental professional students? The objectives are to map the available evidence regarding the impact and evaluation of LS in undergraduate dental education, and to identify gaps in current knowledge. The inclusion criteria were developed using the recommended Population (undergraduate dental professional students), Concept (impact and evaluation of LS) and Context (university, hospital and community-based settings, virtual learning environments) framework. Following a pilot search, the key databases were identified as Medline, Wiley Online Library, Education Full Text, ERIC, Academic Search Complete, Web of Science and Scopus. Sources for unpublished evidence were established. The key journals to be hand searched were listed. No date or language limitation was set. The evidence selection process has been outlined, with two independent reviewers performing title and abstract screening, review of full-text studies, followed by data extraction using a standardised template. The data presentation approach has been specified as a narrative summary accompanied by tabular and/or charted data.

Conclusions
Development of this protocol has provided a comprehensive plan for the conduct and transparent reporting of the subsequent scoping review.

Undergraduate Teaching and Assessment Methods in Dental Implants Curriculum
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Objectives
To achieve consensus amongst an expert panel of prosthodontics/restorative dentistry academics on the best undergraduate teaching and assessment methods of dental implants (DI), and to develop recommendations regarding these methods.

Methods
Semi-structured electronic questionnaires were used to collect data from senior clinical academics involved in the teaching of DI on three consecutive occasions (Delphi method). Round 1 questionnaire asked the experts’ opinions on the best teaching and assessment methods in the undergraduate DI curriculum. In later rounds, panellists were invited to consider their previous responses in light of the overall group response to bring the panel to a consensus. The group response was summarized using simple descriptive statistics, and the target level of consensus for each question was set at ≥ 70%. A response rate of at least 70% between rounds was deemed appropriate to maintain rigour.

Results
Twenty-three senior academic experts from eleven countries agreed to participate. Eighteen (representing nine different countries) completed it in its entirety (response rate 78.3%). After 3 iterative rounds, 45 out of 50 statements obtained the experts’ consensus (90%). 38 of these were on “preclinical and clinical teaching and assessment”, whereas the remaining were on “the minimum competency level”. The total level of consensus was 17.1% (7 statements) and 44.0% (22 statements) in Rounds 1 and 2, respectively. Experts’ consensus was that it is important for students to have a dedicated preclinical course in DI restoration (prosthetic part), while having a preclinical surgical course was not considered important. Also, a minimum competence in DI is not needed for graduation but it is desirable.

Conclusions
The majority of the DI teaching and assessment methods statements achieved consensus during Round 3, reflecting the wide variation among the experts’ opinions. These consensus statements could be considered as guidelines and recommendations to improve future undergraduates’ curriculum in DI.
Occlusal Devices in France: an Assessment of Professional Practice

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Objectives Whether recommendations for the use of occlusal devices are made uniformly in terms of indications, designs, and wearing time is unclear. Different recommendations may lead to different clinical outcomes. The purpose of this survey was to assess the professional practice of dental surgeons in France regarding the use of occlusal devices.

Methods A 26-question cross-sectional survey was sent to a panel of French dentists via the County Councils of the Dental Order. The questionnaire concerned the amount of occlusion-related treatment, the use of an anterior deprogramming device, stabilization splint, and anterior repositioning appliance, and the patient follow-up as well as the drawbacks of using an occlusal device as a therapeutic solution. The statistical tests used in the study were the chi-square test and the Yate correction for continuity.

Results A total of 771 responses were received. Invasive options were still reported as being used as a first-line treatment for temporomandibular disorder, although a statistical difference was found between experienced and recently graduated practitioners, with recent graduates preferring noninvasive options as first-line treatment. Also, the results showed that anterior deprogramming devices were not used or that their application, particularly the length of treatment, was unfamiliar to practitioners. The anterior repositioning appliance seems to be used, but only a few practitioners integrate it into their practice. In general, about one-third of dentists appear to have a good knowledge of occlusion-related practice, in particular, the use of occlusal devices.

Conclusions The results indicated that only 20% to 30% of practitioners have good knowledge of contemporary occlusion-related practice. There is a need for the standardization of practice and improved education for practitioners in the use of occlusal devices.

Evaluation of the Perceived Stress in Final-Year Dental Students

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Objectives Stress is one of the facts of daily life that cannot be ignored and individuals are confronted with many stress factors in their lives. Students are highly vulnerable to mental health issues during the final year faculty of dentistry, and research shown that perceived stress and mental health problems have increased. The aim of this study was to reveal the prevalence of perceived stress among final-year dental students and to explore relationship between stress levels and oral health quality of life (OHQoL).

Methods A total of 102 final-year dental students at the Faculty of Dentistry, Marmara University, Istanbul, Turkey have participated to the survey including Perceived Stress Scale (PSS), OHQoL and a socio-demographic features (age, gender, smoking, education and economic level of parents). Data were analyzed using IBM SPSS Statistics-Version 21 and analyzed using chi-squared test ($X^2$).

Results The study group of the current research is participated of a total 102 final-year dental students, of whom 62 (61%) were female and 40 (39%) were male whose ages range from 20 to 25. The mean perceived stress score was 11,34± 6,52. The stress level of the students 64 (62,7%) was reported to be high. The majority of the students 69 (67,6%) stated high level that oral health related quality of life but no statistically significant difference was found with the level of stress they perceived ($p=0,001$). In addition no difference was found between the genders and stress levels of the students ($p=0,328$). 32 students (31,3%) smoke and it was not found to be related to the perceived stress level ($p=0,520$).

Conclusions The study shows that students’ stress levels during the final-year is at high risk. These findings may not be generalisable nationally as students sampled were enrolled in a single faculty. However Dental Schools should develop stress management programs to enhance stress coping skills of students.
**Machine Learning in Dentistry: Systematic Review**

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**Objectives** Dental conditions are highly prevalent and machine learning (ML) is increasingly used for their prevention, detection, and management. We aimed to systematically compile and synthesize studies using ML in dentistry, and assess their risk of bias and reporting quality.

**Methods** Dental studies employing ML published from January 2015 - May 2021 in Medline, IEEE Xplore, and arXiv were screened. All original studies related to dentistry and without gross reporting fallacies were included. Studies were stratified by ML task (classification, object detection, semantic segmentation, and instance segmentation) and clinical field of dentistry. Temporal and geographical publication trends, methodology, and results were summarized. Risk of bias and adherence to reporting standards were evaluated via QUADAS-2 and TRIPOD, respectively.

**Results** Out of 183 identified studies, 169 were included, all describing various kinds of ML tasks, models, input data, data sources, strategies to generate reference tests, performance metrics, etc. Annual frequency of publications on ML in dentistry steadily increased from 1 (2015) to 68 (2020). Most studies used single-source, private datasets, and the reference test was usually established by professional experts. Classification tasks were most common. Of the studies employing deep-learning models, CNNs were the most frequent architectures. 39 different metrics were used to evaluate model performances, with accuracy, sensitivity, precision, and intersection-over-union being the most common. ML models generally showed high performance (>0.70) across most metrics in each subgroup. However, external validation was performed by only 20% of the studies. Considerable risk of bias and poor adherence to reporting standards was observed.

**Conclusions** ML has been employed extensively, using a wide range of methods, in dentistry. Given the heterogeneity in methodology and reporting, a minimum (core) set of outcome metrics is needed to facilitate comparisons across studies. The overall body of evidence is limited by the risk of bias and poor reporting quality. Description of performance metrics used in the included studies, stratified by type of machine learning task and clinical task

<table>
<thead>
<tr>
<th>Machine learning task</th>
<th>Classification task</th>
<th>Object detection task</th>
<th>Semantic segmentation task</th>
<th>Instance segmentation task</th>
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<tbody>
<tr>
<td>n</td>
<td>58</td>
<td>7</td>
<td>19</td>
<td>7</td>
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<tr>
<td>Model architecture</td>
<td>CNN</td>
<td>Faster R-CNN</td>
<td>Fully CNN</td>
<td>Mask R-CNN</td>
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<tr>
<td>Mean accuracy</td>
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| Oral medicine          | 0.86 (n=9)           | 0.83 (n=2)            | -                         | -                         |
| Oral radiology         | -                    | -                     | 0.98 (n=1)                |                           |
| Orthodontics           | 0.81 (n=6)           | -                     | -                         |                           |
| Oral surgery and implantology | 0.95 (n=3) | -                     | -                         |                           |
| Periodontology         | 0.84 (n=4)           | 1 (n=1)               | -                         | 0.92 (n=1)                |
| Prosthodontics         | 0.99 (n=2)           | -                     | -                         |                           |
| Others                 | 0.94 (n=13)          | 0.99 (n=1)            | 0.98 (n=3)               | 0.98 (n=2)               |

**Description of performance metrics used in the included studies, stratified by type of machine learning task and clinical task**
<table>
<thead>
<tr>
<th></th>
<th>Restorative dentistry and endodontics</th>
<th>Oral medicine</th>
<th>Oral radiology</th>
<th>Orthodontics</th>
<th>Oral surgery and implantology</th>
<th>Periodontology</th>
<th>Prosthodontics</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean average precision</td>
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<td>0.90 (n=1)</td>
<td>-</td>
<td>-</td>
<td>0.91 (n=2)</td>
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<td>0.98</td>
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<td>(n=1)</td>
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<tr>
<td>Mean intersection over union or DICE or Jaccard indices</td>
<td>-</td>
<td>-</td>
<td>0.73 (n=1)</td>
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<td>0.88 (n=1)</td>
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<td>0.95</td>
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<td>0.93 (n=1)</td>
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<td>0.88 (n=1)</td>
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<td></td>
<td>0.95 (n=1)</td>
<td>-</td>
<td>0.80 (n=6)</td>
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CNN, convolutional neural network; R-CNN, recurrent convolutional neural network
**Students’ Fingers Power Regulation is Affected by Phantom Course Training**

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**Objectives**

People who work with rotating devices report a decrease in hand sense touch and a decrease in grip force of the fingers or palm. **Aims:**

1. To examine the relationship between the palm’s anthropometric measures and the level of accuracy in power regulation.
2. To examine the change in the level of accuracy after intensive training in the phantom course.

**Methods**

Participants: 30 dental students in their phantom laboratory course, generally healthy, with normal or corrected vision, right or left-hand dominance. Each subject underwent anthropometric measurements of the palm and wrist. Subjects performed accuracy tests by tracking force magnitude-related paths on a computer screen using the fingers’ force modulation. Tests were performed tests with the dominant and non-dominant hands twice: T0-3 months after commencing the lab, T1-5 months later. The accuracy was calculated by the RMS of the tracking line from the original one.

**Results**

The accuracy of force modulation for the dominant hand and the non-dominant hand were similar at both measurement times (p=0.19). However, at T1 the level of the accuracy decreased (RMS error increased) in the dominant and non-dominant hands in all force modulation tests. A significant decrease (p<0.04) was found in the force modulation accuracy after training in the phantom course in most tests in the dominant hand. This significant decrease was not found in the non-dominant hand. No significant association was found between accuracy in force modulation in the various tests and anthropometric palm and wrist measures.

**Conclusions**

Intensive training using rotatory tools reduces the ability of force modulation of non-dental tasks. This deterioration is not related to anthropometric measurements of the hand.

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**Knowledge and Self-Confidence of Dental Students About Implants: Online Survey**

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Selçuk University, Konya, Turkey

**Objectives**

The use of dental implants for the prosthetic treatment of edentulous or partially edentulous individuals has increased rapidly since the adoption of the concept of osseointegration. Implant surgery is a delicate procedure that requires knowledge and experience. For this reason, in our study, we aim to evaluate the level of knowledge and self-confidence of dental students and newly graduated students who will perform these treatments in the future.

**Methods**

As the study population, 4th and 5th year dental students and newly graduated dentists who were targeted. The questionnaire consists of 15 questions in total to evaluate their gender, class information and, their approach to implant treatments. Students were invited with open invitation posts in various social media applications. SigmaPlot program was used for statistical evaluation.

**Results**

A total of 259 participants, 157 women and 102 men, who answered all fifteen questions included in the study. When the level of knowledge they felt about implant procedures was compared between male and female students, the level of feeling informed by male students was higher (p:0.024). When the comparison was made between students in different classes, no significant results were found (p:0.873). When the level of knowledge that students felt about implant procedures at graduation was compared, there was no significant difference between male and female students (p:0.507). A statistically significant difference was found in the gender-based evaluation of the need for specialists to implant surgery (p<0.001). No significant difference was found when the same question was compared between the classes (p:0.052).

**Conclusions**

The level of knowledge they felt about implant procedures and self-confidence among dental students and newly graduated dentists are poor. To prevent this, the number of theoretical and practical courses on implants can be increased.
M101, an Oxygen Carrier Derived From Arenicola Marina, Ameliorates Bone Healing

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1Dental Faculty, Strasbourg, France, 21260, INSERM, Strasbourg, France, 3Lutech, SATT, Paris, France, 4Hémarina, Morlaix, France

Objectives M101 is a natural extracellular hemoglobin isolated from the marine worm Arenicola marina with exceptional oxygenation properties. Recently, it has been demonstrated that M101 hydrogel could improve periodontal healing through increase of O2 level, anti-inflammatory and antibacterial properties. The aim of this study was to evaluate the potential of a hydrogel loaded with M101 to promote bone healing.

Methods A 5mm calvarial defect was induced in mice (n=6/group) with a bur and lesions were treated with 50 μL of hydrogel (hyaluronic acid + xanthan) containing M101 (1g/L and 2g/L). The healing of the bone lesion was evaluated by histomorphometric analysis (hematoxicillin/eosin and red alizarin staining) and micro-CT after 1 month. Neobone formation was appreciated by immunofluorescence (BSP2 and osteocalcin expression). Transmission electron microscopy was performed to evaluate the presence of M101 at the injection site.

Results After 1 month of healing, treatment with M101 loaded hydrogel improved defect closure. The surface of neobone area was significantly improved in mice treated with M101 gel (1mg/L and 2 mg/L) vs placebo gel and natural healing (p<0.05). Interestingly, the bone healing in M101 treated mice was associated with an increased fibrous tissue height. The neobone formation was associated with BSP2 and osteocalcin expression. Electron microscopy revealed that, after 1 month, M101 was no more detected at injection site confirming its elimination at injection site.

Conclusions This study demonstrated that hydrogel loaded with M101 could be of interest to treat infrabony defects or bone lesions. However, there is a need to evaluate more precisely the impact of M101 on osseous cells and tissue for periodical regeneration.

Non-Surgical Periodontal Therapy Ameliorates Dysbiosis in Children With Primary Immunodeficiencies

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Objectives Primary Immunodeficiencies (PID) are a heterogenous group of immune dysfunctional disorders arising from rare genetic defects affecting humoral and cellular immunity, which can lead to reduced ability to control dental plaque. This study aimed to characterise the subgingival plaque microbiome of gingivitis or periodontitis in neutropenic PID children compared to healthy controls, and assess if there is a positive host response to non-surgical periodontal therapy.

Methods Children diagnosed with PID neutrophil defects (n=24) and age matched systemically healthy control participants (n=25) were recruited from Great Ormond Street Hospital and the Paediatric Dental Department, Royal London Hospital according to an ethics approved protocol. Subgingival plaque was collected from 3 first molars and 1 first incisors at baseline and 6 months, post non-surgical periodontal therapy. DNA from samples was extracted and the 16S rRNA gene V1-V2 region sequenced using Illumina 2x300bp paired end method. The sequences were processed and analysed via the DADA2 amplicon sequence variant pipeline.

Results Significant shifts in the community structure was observed at the 6-month follow up, post therapy with decrease in alpha and beta diversities as measured by the Shannon and Simpson indices. An increase in relative abundance of health associated species such as Rothia spp. and Lautropia spp. were observed in the PID children at follow up compared to baseline, suggesting a positive host response to treatment in line with improvements observed with the clinical assessments. At baseline, species such as Granulicatella spp, were positively associated with blood neutrophil counts of the PID children highlighting the host-microbial relationship that exists in the oral cavity.

Conclusions Non-surgical periodontal therapy was found to help ameliorate dysbiosis in gingivitis and periodontitis of neutropenic children with PID disorders. Characterising the subgingival microbiome in PID children has revealed novel host-microbial relationships that may be important in the oral microbiome.
OMVs of P. gingivalis as Therapeutic Targets for Human Antibodies
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Objectives Chronic periodontal disease, once established, is irreversible. Deep within diseased pockets resides major virulent microorganisms including the notorious red-complex trifecta of Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia. These have become reliably successful in initiating and sustaining destructive inflammation within the periodontium owing to their surface-associated and soluble virulence factors. Amongst them, Porphyromonas gingivalis (P. gingivalis) has proven to be the most important not only for local disease severity but also for remote systemic pathologies. As a highly successful keystone periodontal pathogen, P. gingivalis produces potent virulence factors selectively packaged into outer membrane vesicles (OMVs). OMVs diffuse out of dysbiotic dental biofilms to orchestrate major offensive and ‘defensive’ events locally and remotely. Their in-vivo presence triggers a heightened anti-OMV humoral immune response which, we hypothesize, may provide protection against local tissue injury and systemic complications of chronic periodontitis.

Methods In this study, we harnessed the diversity and specificity of the human antibody response in constructing a panel of IgG-type single-chain variable fragment (scFv) (VH-VL orientation) molecules against OMVs of P. gingivalis. Utilizing molecular ‘gene-expression’ machines within a rabbit reticulocyte system, mRNA transcripts and nascent peptides of linked antibody VH-VL domains were coupled, creating a large library of stable post transcriptional-translational complexes, and selected for binding to OMVs.

Results Following purification, scFv binders were further analysed in immunological, anti-haemmaglutination and anti-growth assays to demonstrate binding and neutralization of OMV-associated antigens.

Conclusions In total, out of over 590 panel constructs, we have successfully identified 15 unique binders of the IgG/lambda and IgG/kappa light chain series, with preliminary in-vitro experiments suggesting their potential as diagnostics, therapeutics, and research instruments. Currently, the most compelling candidates are being re-engineered into full-length glycan-modified IgG molecules.

Machined Fiberglass Periodontal Retainers
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Objectives The reduction in periodontal tooth mobility using retainers restores the comfort of our patients and improves the results of our treatments, particularly surgical ones, during procedures for regeneration of infra-bony defects. However, their realization can be complex: the conventional impressions of mobile teeth are risky (trauma), imprecise and source of stress for the patient and the practitioner. These devices thus regularly debond or may break. The objective is to show through several clinical cases an effective method of retainers with a digital work-flow.

Methods A digital impression is taken using an optical camera (Omnican, Sirona®). The STL file is transmitted to the prosthetist and the machines milled a retainer using a block of glass fibers (Numerys GF, Itena) which is composed of 80% unidirectional radiopaque glass fibers and 20% epoxy resin. The glass fiber blocks have a flexural strength of 990MPa and a modulus of elasticity of 23.8GPa close to dentin. The retainer is bonded under a sealed operating field (rubberdam) using an MR2 type bonding protocol. Excess of bonding material is removed with a silicone brush and the bonding joint is polished.

Results The digital impression allows optimal precision of the retainer on the teeth. Several cases are presented with at least one year of clinical follow-up. The retainers show no detachment or fracture.

Conclusions Reducing tooth mobility improves the functional comfort of our patients and optimizes the results of our non-surgical and surgical treatments. Retentions machined in fiberglass and made from an optical impression make it possible to obtain precision and ideal mechanical properties. However, retention remains a tool and not an etiological treatment of periodontal disease.
**Effects of Periodontal Treatment on MiRNAs Expression in Periodontitis Patients**

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**Objectives** This study aimed to assess the effects of periodontal treatment of microRNAs (miRNA) expression in gingival crevicular fluid (GCF) in patients with periodontitis (PT) and cardiovascular disease (CVD) as early disease markers of CVD risk reduction.

**Methods** Through a 6-months prospective-controlled clinical trial design, were recruited healthy subjects (n=18) and patients with PT (n=18), CVD (n=19) and PT+CVD (n=19). Patients were assigned to receive a session of full-mouth Scaling and Root Planing (FM-SRP) protocol and were regularly monitored for clinical and GCF miRNA-7a, -21, -100, -125b and -200 changes at baseline, 60 and 180 days follow-up. The primary outcome was the evaluation miRNA changes in GCF evaluated by real-time PCR. A multivariate generalized linear model was used to identify the most significant predictors at baseline that influenced miRNA variation changes after 6-months of FM-SRP.

**Results** At 3 and 6 months after therapy, FM-SRP treatment significantly reduced each analyzed periodontal parameter in all groups. Compared with healthy subjects, patients with PT, CVD and PT+CVD presented significant dysregulated miRNAs GCF expression. FM-SRP produced, at -3 and at 6-months after therapy, a significant reduction of the miRNA 7a-5p, 21-3p, 21-5p, 200b-3p, and 200b-5p, and a significant increase in the miRNA 100-5p and 125-5p (p<0.001). The multivariate analysis evidenced that periodontitis at baseline was a significant predictor of the decrease of miRNA 7a-5p (p=0.016) and miRNA 21-3p (p<0.001). Moreover, presence of periodontitis (miRNA 100-5p, p=0.004; miRNA 200-5p, p=0.019) and CVD at baseline were significant predictors of the increase of miRNA 100-5p (p<0.001) and the decrease of miRNA 200-5p (p=0.017) at 6-months after FM-SRP.

**Conclusions** After 6-months follow-up, FM-SRP periodontal treatment yielded a significant reduction of miRNAs 7,-21,-200 and the upregulation of miRNA-100 and -125. The presence of periodontitis at baseline was a significant predictor, after 6-months of therapy, of GCF miRNA expression changes associated with early augmented CVD risk.
Osteogenic Potential of Doxycycline Doped Collagen Membranes

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Objectives To evaluate the effect of collagen membranes doped with doxycycline and dexamethasone on the proliferation and differentiation potential of osteoblasts in vitro.

Methods Bovine-derived collagen membranes (Symbios®, Dentsply Sirona) were doped with doxycycline (Dox) or dexamethasone (Dex). Established groups: 1) Undoped membranes (Col-M) (control), 2) Dox functionalized membranes (Dox-Col-M) and 3) Dex functionalized membranes (Dex-Col-M). The membranes were subjected to MG63 osteoblast-like cells culturing (ATCC, Manassas, VA, USA) during 48h. Proliferation was assessed by MTT-assay and differentiation by alkaline phosphatase activity (PA), real-time quantitative polymerase chain reaction (RT-qPCR) to study the expression of Runx-2, OSX, ALP, OSC, OPG, RANKL, Col-I, BMP-2, BMP-7, TGF-β1, VEGF, TGF-βR1, TGF-βR2, and TGF-βR3. Field Emission Scanning Electron Microscopy was also performed to assess the osteoblasts morphology. Mean comparisons were conducted by one-way ANOVA and Tukey tests, qPCR results were evaluated by non-parametric tests (p < 0.05).

Results Osteoblasts proliferation was significantly enhanced by membranes doping, following the trend Dox-Col-M (0.44) > Dex-Col-M (0.32) > Col-M (0.23). PA activity was significantly higher on osteoblasts cultured on Dox-Col-M (0.95 IU), than in the others groups (0.11 IU). Runx-2, OSX, ALP, OSC, BMP-2 and BMP-7 were overexpressed (3-fold) on osteoblasts cultured on Dox-Col-M if compared to the control group. TGF-β1, VEGF, TGF-βR1, TGF-βR2, and TGF-βR3 were 2-fold overexpressed in Dox-Col-M if compared to the control. Moreover, Dox-Col-M down-regulated (4-fold) RANKL expression if compared to Col-M. Furthermore, in FESEM analysis, osteoblasts on Dox-Col-M appeared to produce more osteoid substance and showed more elongated morphology; events that have been associated to more advanced proliferative and differentiation state.

Conclusions The functionalization of collagen membranes with Dox, produced an increase in the proliferation and differentiation of osteoblastic cells, suggesting a potential therapeutical application in guided bone regeneration processes. Supported by Ministry of Economy and Competitiveness and European Regional Development Fund [PID2020-114694RB-I00 MINECO/AEI/FEDER/UE]. M.T.-O. is fellow FPU of Ministry of Universities [FPU20/00450].
Cell Secretome-Based Strategy for in Vivo Guided Bone Regeneration

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Objectives In complex bone defects, conventional guided bone regeneration (GBR) approaches are not fully predictable. In such cases, supplementing biomaterials with mesenchymal stromal cells (MSC) or growth factors, e.g. leukocyte-platelet rich fibrin (L-PRF), has been proposed to enhance GBR outcomes. Recently, cell secretomes have emerged as a promising alternative to cell transplantation and growth factor-concentrates. Based on its paracrine effects, MSC conditioned media (CM) has been proposed as a simpler and more cost-effective strategy for bone regeneration. However, the optimal mode of CM delivery is unknown. The objective of this study was to functionalize collagen membranes (MEM) with CM and test their capacity for GBR in rat calvarial defects in comparison to conventional GBR, allogeneic MSC and L-PRF.

Methods Pooled CM was prepared from human MSC and L-PRF was obtained from whole blood (n=3 donors each). Commercial MEM (BioGide, Geistlich Pharma) were functionalized with CM via soaking (CM-soak) or lyophilization (CM-lyo) and applied on 5-mm calvarial defects of Lewis rats; control defects were treated using conventional GBR (native MEM only), MEM with allogeneic rat-MSC (MSC), MEM with PRF-conditioned media (PRF) or left untreated. New bone formation (NBF) was evaluated via in vivo micro-computed tomography (mCT) at 2 weeks, and ex vivo mCT and histology at 4 weeks. mCT and histomorphometric data were analyzed to obtain defect coverage (NBF%) and relative new bone area (NBA%). Data were statistically analyzed at a 0.05 significance level.

Results After 2 weeks, in vivo mCT revealed significantly greater mean NBF% in CM-lyo (85.5%) vs. CM-soak (21.6%), MSC (18.8%), MEM-only (18.5%), PRF (28.2%) and untreated groups (11.6%; p<0.05). After 4 weeks, only CM-lyo (78.9%) revealed significantly greater NBF than the untreated control group (28.9%; p<0.05), while CM-soak (46.9%), MSC (56%), MEM-only (57.8%) and PRF (48.07%) were comparable (p>0.05). Histology revealed significantly greater NBA% in CM-lyo vs. CM-soak, MSC, MEM-only and PRF groups (p<0.05).

Conclusions CM-lyo promoted earlier and greater NBF in rat calvarial defects compared to conventional GBR, allogeneic MSC and L-PRF. Lyophilization of CM represents an efficient method for MEM functionalization to enhance GBR in advanced bone defects.

In vivo GBR: Quantification of mCT data showing bone coverage (A) and bone volume to total volume ratio (B) at 2 and 4 weeks. (C) Histomorphometric data showing total new bone, new bone with and without embedded membrane fibres and remnant collagen membrane. * indicated statistically significant differences (p<0.05)
Single-Cell RNA Sequencing of Fibroblasts From the Oral Mucosae
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Objectives To characterize the gene expression, variability and cell populations of fibroblasts of the oral lining and masticatory mucosa.

Methods Primary human fibroblasts were retrieved from the posterior masticatory hard palate and the lining alveolar mucosa of three individuals. Single-cell gene expression profiles were obtained using 10X genomics Single cell RNA sequencing (scRNA-seq).

Results Uniform Manifold Approximation and Projection (UMAP) clustering clearly distinguished between fibroblasts of masticatory or lining mucosa that formed distinct clusters (Figure 1). Interestingly, the intra-individual variability was significantly smaller than the tissue-origin variability, with significant cluster overlap between different individuals, but not between masticatory and lining fibroblasts. Differences in specific gene groups and pathways were observed between fibroblasts from the two tissues. These largely agreed with previously published bulk data and included genes for extracellular components like structural collagens and cranial neural crest markers. Within each tissue, distinct clusters or subpopulations were annotated using SingleR. The annotations were taken from Human Primary Cell Atlas (HPCA). These showed differential expression in multiple genes, including genes regulating the extracellular environment. Interestingly, a single cluster expressed multiple mesenchymal stem cell (MSC) markers (Figure 2). This cell cluster was located at the intersection of the two tissues but contained predominantly cells of masticatory mucosa origin.

Conclusions Our previous work has demonstrated that fibroblasts from the lining and masticatory oral mucosae are phenotypically heterogeneous. Here, we extend these findings to show that these changes are not the results of differences in averages, but of two distinct cell populations. Moreover, these cells form distinct clusters, showing differential expression of genes coding to secretory proteins and regulators of the extracellular matrix. Finally, cells expressing MSC genes are enriched in masticatory mucosa. These clusters may contribute to specific physiological functions and have relevance for potential therapeutic applications.
Osteogenic Profile of Mechanically Stimulated Mesenchymal Stem Cells
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Objectives The transplantation of mesenchymal stem cells (MSC) is a promising alternative to conventional bone augmentation strategies. Although pre-conditioning of MSC towards osteogenic lineage by mechanical stimuli has been suggested to further improve bone regeneration, the mechanically-induced osteogenic profile has not been well described. The aim of the study was therefore to identify the osteogenic property of MSC under mechanical stimulation and then explore the role of cytoskeletal adaptation to the stimuli in mechanically-enhanced osteogenic properties.

Methods Bone marrow-derived MSC from rat femurs were seeded on 3D polymeric scaffolds and subjected to fluid flow in a bioreactor system. Their osteogenic profile was evaluated by screening mRNA expression pattern, alkaline phosphatase (ALP) activity, and calcium deposition on days 7 and 14. Furthermore, small molecule compounds inhibiting cellular morphogenesis were applied to evaluate the alteration of osteogenic properties under the stimulation.

Results After 7 days of the dynamic culture, 29 out of 85 selected putative osteogenic markers were upregulated, which included key transcription factors for osteogenesis, Runt-related transcription factor 2 (RUNX2) and Sp7 Transcription Factor (SP7), Bone morphogenetic proteins (BMPs), Fibroblast growth factors (FGFs), and collagen family. This trend remained nearly unchanged after 14 days of the culture. Interestingly, the osteogenic profile differed from that was pharmacologically induced by the conventional osteogenic cocktail (i.e., dexamethasone, beta-glycerophosphate, ascorbic acid), in which 28 and 52 markers were upregulated on days 7 and on days 14, respectively. The upregulated expression of the osteogenic markers by fluid stimuli was disrupted by small molecule compounds inhibiting cell morphogenesis. Similarly, enzymic activity of ALP was significantly enhanced in the mechanically-stimulated MSC, which was inhibited by the compounds. Alizarin red S staining confirmed that the MSC under fluid flow deposited calcium nodes on the scaffolds greater than MSC in a static environment but significantly less than the pharmacologically induced counterpart.

Conclusions Our data suggests that mechanically-induced osteogenesis is distinct and driven by cellular morphological adaptation to the dynamic environment.
Mouse Tooth Organoids: Expanding the Toolbox of Dental Regenerative Medicine
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Objectives Worldwide, tooth loss, typically a consequence of oral or congenital disease or traumatic injury, is an important health problem, commonly treated by implantation of synthetic materials. Despite considerable research efforts using different stem cells and biomaterials, complete regeneration of dental tissues is not yet possible. Being able to induce tooth regeneration with biological material, preferentially of biological origin, would resolve most of the limitations of current treatments based on synthetic components. Organoids, self-organizing stem cell-derived three-dimensional in vitro reconstructions of an organ, provide a powerful strategy to meet this objective.

Methods Here, we embarked on the establishment of mouse tooth organoids as a tool to explore tooth biology and regeneration.

Results We successfully established organoids from both early-postnatal mouse molars and incisors. The organoids were found long-term expandable, at present for more than 10 passages. Gene expression and immunostaining analyses revealed the presence of multiple dental markers, including amelogenin (AMELX) and known dental epithelial stemness markers (i.e., SOX2, INTA6) were detected in the tooth organoids suggesting a prominent stemness character. Remarkably, incisor organoids showed a strong requirement for EGF supplementation, which was not seen in molar organoids. Currently, we are enhancing the differentiation capacity of the molar- and incisor-derived organoids using in vitro and in vivo approaches. Firstly, we have obtained an improved in vitro protocol resulting in drastic increases in AMELX and ODAM. Secondly, co-culture of organoid-derived dental epithelium with dental pulp stem cells (as so-called ‘assembloids’) further improved ameloblast-like differentiation. Finally, mouse tooth organoids were able to survive for at least 7 days after subcutaneous transplantation in mice – indicating their potential use for regenerative medicine and bioengineering.

Conclusions Eventually, our study will help to unravel molecular and cellular aspects of tooth development, as well as of dental stem cell biology, in the end instrumental for pursuing tooth regenerative replacement therapy.
**Endothelial Expression of ALDH1&2 in Oral Cancer and Leukoplakia.**

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**Objectives** Cancer stem cells (CSCs), are responsible for tumor growth and cancer cell self-renewal and self-proliferation. The immunohistochemical detection of cancer stem cell marker aldehyde dehydrogenase ALDH 1&2 had been previously described in cancer epithelial cells. The aim of our study is the investigation of stromal endothelial cells of ALDH.

**Methods** Tissue samples were acquired from 21 oral squamous cell carcinomas, 30 potentially malignant lesions (Leukoplakia) and 5 from the control group of lesions consisting of histologically healthy epithelium (for example: fibromas). The samples were obtained from the archives of the Department of Oral Medicine/Oral Pathology, Dental School, Aristotle University of Thessaloniki, Greece during the period 2009-2019. The results describe the quantitative immunohistochemical expression of the CSC' biomarker ALDH on protein level through SPSS Pearson Chi-square. The level of statistical significance was set at p value< 0.05.

**Results** The endothelial staining of ALDH was observed in the majority of the OSCC cases (in 13 out of 21 cases), irrespective of the grade of differentiation (well, moderately, poorly differentiated OSCC). ALDH was expressed in 5 cases of OL out of 30 and in none of the control group. In particular, the endothelial staining of ALDH was expressed significantly more in the OSCC group compared to the OL group (p-value<0.01), whereas the expression of ALDH was not statistically significantly altered between the OL group and the control group (p-value =1.000).

**Conclusions** Increased expression of ALDH1 & 2 was observed in endothelial cells in OSCC. This finding is in agreement with findings regarding kidney cancer. In particular, increased expression of ALDH in cancer-associated endothelial cells is reported and positive correlation with resistance in chemotherapy is established. Thus, the immunohistochemical expression of ALDH in cancerous lesions support its role in carcinogenesis and its possible usage as prognostic factor.

**Exosomes of Dental Pulp Cells Cultivated in Hollow Fiber Bioreactor**

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**Objectives** Within the pulp resides a mesenchymal stem cell population, designated as ‘dental pulp stem cells’ (DPSCs) which have regenerative properties in a wide variety of disorders such as ischemic stroke and dental tissue regeneration. Previously, our group demonstrated that DPSCs are able to induce blood vessel growth or angiogenesis in vitro and in vivo. This effect was not only caused by the proteins but also the extracellular vesicles (EVs) produced by DPSCs. EVs are small membrane particles released by cells carrying RNAs, proteins, lipids and have an important role in cell communication. Given the remarkable regenerative properties of these EVs, they have great clinical potential. Unfortunately, this clinical translation is hampered by the poor scalability of EV production, which currently only delivers small batches and low yields.

**Methods** Thus, our goal is to increase the production of DPSC-EVs by a hollow fiber bioreactor (HFB) cultivation to mimic the in vivo microenvironment. DPSC viability was monitored using glucose/lactate measurements. EVs yield was measured using fluorescence nanoparticle tracking analysis (fNTA), while EVs protein profile and structure was analyzed with Western Blot (WB) and transmission electron microscopy (TEM), respectively.

**Results** DPSCs can be cultured in 20 kDa HFB for at least 25 days as they produce lactate during that period. EVs were harvested 2 times a week and fNTA showed that around $10^9$ particles per harvest were produced. Western Blot analysis revealed that EVs were positive for EVs-markers CD9, CD63, and CD81 while ultrastructure analysis demonstrated their typical cup-shaped form.

**Conclusions** We demonstrate for the first time DPSCs cultivation in a 3D HFB setup, while being able to produce EVs. In future experiments, we will further focus on the biological functions of these EV fractions.
**Dental Stem Cells-Mediated Suicide Gene Therapy for Oral Cancer.**

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**Objectives** Oral squamous cell carcinomas (OSCC) are malignancies in the oropharynx and the oral cavity. These tumors are often associated with alcohol and tobacco use as well as with HPV infection. To reduce side effects and save healthy tissue, it is essential to establish more targeted and specific therapeutic strategies. We aim to develop a new therapy for OSCC based on dental pulp stem cell (DPSC)-mediated suicide gene therapy. Hence, we lentivirally overexpress the herpes simplex virus type 1 – thymidine kinase (HSV1-TK) in DPSC using a polycistronic construct co-expressing the imaging reporter gene Firefly luciferase, generating HSV1-TK+ DPSC. Upon phosphorylation by HSV1-TK, GCV turns into its toxic metabolite and induces cell death.

**Methods** The expression of the HSV1-TK gene in DPSC is assessed by polymerase chain reaction (PCR) and immunocytochemistry (ICC). HSV1-TK+ DPSC-mediated OSCC cell killing in a DPSC/OSCC co-culture system is examined over six days by confluency analysis and alamarBlue viability assays. For successful DPSC-based suicide gene therapy, gap junction formation between DPSC and OSCC cells is essential to pass the cytotoxic GCV to the OSCC cells. This is assessed via immunocytochemistry for connexin-43 in the co-culture system.

**Results** HSV1-TK expression is significantly higher in transduced cells compared to their controls, as confirmed using PCR and ICC. Moreover, we successfully demonstrated HSV1-TK+ DPSC-mediated OSCC cell killing in vitro in a DPSC/OSCC co-culture system. The presence of connexin-43 between DPSC and OSCC suggests gap junctional communication between DPSC and OSCC.

**Conclusions** In conclusion, we successfully induced the expression of HSV1-TK in different DPSC patient lines. Additionally, our results suggest that gap junctional intercellular communication is responsible for DPSC-mediated OSCC cell killing. These data show that DPSC have a high potential to be used as a novel targeted therapy for OSCC, which should be confirmed in further studies.

**Condensed Adipose Tissues Membrane as Potential Implants to Bridge Soft Tissues**

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**Objectives** Tissue engineering, aimed at producing fully functional tissue replacements, has provided promising alternatives for regenerating soft and hard tissue defects. Adipose tissues, which are abundant in the body, are readily accessible through liposuction or from the buccal or abdominal fat pads. These tissues are an excellent source of mesenchymal stromal cells (MSCs) and growth factors. Yet, extensive clinical use of these tissue sources as biological tissue grafts has yet to be reported. We have recently developed a novel technique to prepare adipose-derived membranes with tissue regeneration potential, able to withstand mechanical forces, and undergo tissue integration after implantation.

**Methods** Adipose tissues were harvested from 350-400 gr rats and from healthy human subjects undergoing abdominoplasty and processed using a prototype apparatus for adipose tissue condensation. Condensed adipose tissue membranes from rats were used for a soft tissue implantation model. Condensed adipose tissues from humans were tested for their mechanical properties, cellular sprouting and presence of MSCs. Condensed tissues were compared to uncondensed controls.

**Results** In vivo implantation of rat membranes indicated rapid perfusion and integration of condensed membranes opposed to uncondensed controls. Condensed membranes from human specimens exhibited superior mechanical characteristics and cellular sprouting, later characterized as stromal cells able to undergo differentiation toward adipose and osteogenic lineages. Preliminary randomized clinical trials of a human Buccal fat pad have demonstrated significant superiority of soft tissue healing.

**Conclusions** Preliminary results indicated that a rat in vivo model can be used to assess the contribution of adipose tissue condensation to defect bridging and remodeling. Moreover, results from human specimens indicate the possibility of clinical translation.
Bonding Hydrophobic 2-Step Universal Adhesives to Low C-Factor Flat Dentin

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Objectives
To investigate water sorption of experimental 2-step universal adhesives (UAs) providing a particle-filled hydrophobic adhesive resin with a certain film thickness and to determine whether these formulations help to achieve durable bonding effectiveness to low C-factor flat dentin.

Methods
Three experimental 2-step UAs, combining a 10-MDP-based primer with hydrophobic adhesive resins differing only for filler, referred to as BZF-21 (bioglass filler), BZF-29 (silica filler), and BZF-29_hv (higher silica filler loading), all prepared by GC, along with the commercial adhesive Clearfil SE Bond 2 (C-SE2, Kuraray Noritake) were investigated. Resin disks (15-mm diameter, 1-mm height) of the four adhesives were immersed in distilled water at 37°C for 1 week, 6 months and 1 year to measure water sorption (n=5). The ‘immediate’ and ‘aged’ (6-month/1-year water-storage) micro-tensile bond strengths (µTBSs) of the adhesives, applied either in etch-and-rinse (E&R) or self-etch (SE) mode using a split-tooth design to low C-factor flat dentin, were measured (n=10). Statistical analyses were performed using a linear mixed-effects (LME) model and Kruskal-Wallis test (p<0.05).

Results
Lower water sorption was recorded for all UAs compared with C-SE2. The µTBSimmediate of BZF-21 and BZF-29 was not significantly different from that of C-SE2. After artificial aging, the µTBS1-year of all two-bottle UAs was significantly lower than that of C-SE2, except for BZF-29 when applied in E&R mode. Significant reduction in µTBS upon 1-year aging was recorded for BZF-21 and BZF-29, except for BZF-29 applied in SE mode. Significant difference between E&R and SE bonding modes was found for all adhesives except BZF-21.

Conclusions
Among the three experimental UAs, BZF-29 revealed the most comparable bonding performance to C-SE2 in a low C-factor condition (flat dentin).

Ionic Bonding Potential of new Methacrylamide Functional Monomers

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Objectives
10-methacryloxydecyl dihydrogen phosphate (10-MDP) is known as one of the today’s most effective functional monomers to bond to dentinal HAp. However, 10-MDP has been documented to still be sensitive to hydrolytic degradation. This study aimed to test if new methacrylamide functional monomers with improved hydrolytic resistance and different spacer-chain lengths possess at least the same ionic bonding potential as 10-MDP.

Methods
Three methacrylamide functional monomers (ALP), being referred to (based on chain length) ‘ALP6’, ‘ALP8’ and ‘ALP10’ and synthesized by Dentsply Sirona, were investigated for the solubility of their Ca-salts using ICP-AES and XRD, and their chemical interaction with dentin using TF-XRD. Furthermore, the contact angle (CA) of water on ALP-treated dentin was measured to determine the surface hydrophobicity/hydrophilicity. Finally, the ALP-treated dentin surfaces were ultrastructurally investigated for potential nano-layering formation with TEM. The functional monomer 10-MDP (Dentsply Sirona) served as reference.

Results
Ca_ALP6 could not be prepared. Ca_10-MDP was least soluble, while Ca_ALP8 was most soluble. 10-MDP revealed the highest CA (92.3), while ALP6 appeared most hydrophilic (lowest CA: 20.2). TF-XRD disclosed the highest nano-layering intensity for 10-MDP. TEM revealed nano-layering formation for 10-MDP and ALP10, but not for ALP6 and ALP8.

Conclusions
ALP8 and ALP10 revealed less stable Ca-salt formation and lower nano-layering potential. 10-MDP remained the most effective functional monomer in terms of stable Ca-salt formation, nano-layering and durable chemical bonding potential to dentin and HAp.
O197
Dentin/Enamel Shear-Strengths and Failure-Modes of Self-Etch and Universal Bonding Agents.
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Objectives The aim was to measure shear strengths and failure modes of three ‘universal’ self-etch and two self-etch bonding agents to bovine dentin and enamel, following adhesive application by inexperienced operators and to evaluate the shear strength data with and without exclusion of cohesive failures.

Methods Experiments were performed by 4 cohorts of undergraduate dental students (60 students, 2017-2020). Bond strengths of three self-etch ‘universal’agents: OBU (Optibond Universal, Kerr; PBA, Prime&Bond active, Dentsply-Sirona); SBU (Scotchbond universal, 3M) and two self-etch bonding agents: XS, (Xeno select, Dentsply-Sirona); OBXTR, (Optibond XTR, Kerr) were evaluated. 1080 composite-cylinders were bonded perpendicularly to sectioned bovine dentin and enamel surfaces. Shear-strengths were measured (24 hours post-bonding).

Analysis of overall data was made via mixed-mode ANOVA. This was repeated with cohesive failures excluded.

Results OBU and OBXTR showed comparable dentin and enamel bond strengths, whereas lower strengths were found on enamel for SBU (p<0.001) and PBA (p=0.015). (Fig.1 cohesive and adhesive failures pooled). For all adhesives, more cohesive failures were found in dentin than in enamel (Fig.2). SBU exhibited most cohesive failures in dentin, followed by OBXTR and PBA. In Fig.3, shear bond strengths are depicted of specimens with adhesive failures only (i.e. cohesive failures excluded).

Conclusions For OBXTR higher shear strengths were measured with cohesive fractures. After exclusion of cases with cohesive fracture, this material fell behind other materials in the sequence of average shear strengths. This did not reflect the actual performance of the material. Therefore, in statistical analysis we do not recommend exclusion of data based on fracture behavior.

Fig. 1 - Shear strength of bondings on enamel and dentin (all data included, i.e. adhesive and cohesive failures)

Fig. 2 - Percentage of adhesive and cohesive failures
Objectives The aim of this in-vitro study was to compare the microtensile bond strength ($\mu$TBS; MPa) of two universal adhesives in etch-and-rinse mode with a two-step etch-and-rinse adhesive to sound (SD) and caries-affected (CAD) dentin.

Methods 18 extracted human third molars with occlusal caries were ground flat until dentin was exposed. Caries-infected dentin was removed with 600-grit SiC paper and CAD was identified using visual, tactile and caries-detecting dye methods. Then the teeth were randomly divided into three groups ($n=6$) according to the tested adhesives; Adper Single Bond 2 (SB; 3M ESPE), Prime&Bond Universal (PBU; Dentsply, Sirona) and G-Premio Bond (GP; GC). All the adhesives were applied in etch-and-rinse mode and composite buildups were done with a microhybrid composite (Z250, 3M ESPE). Following storage for 24 h at 37°C in distilled water, the bonded specimens were sectioned into resin-dentin sticks and tested in a universal testing machine (Instron) at 0.5 mm/min. Data were analyzed using two-way ANOVA and post-hoc Tukey’s tests ($p<0.05$).

Results $\mu$TBS was significantly influenced by the type of the adhesive ($p=0.004$) and the dentin substrate ($p=0.0001$). Bonding to SD was significantly higher than to CAD for all the adhesives ($p<0.05$). GP showed significantly lower $\mu$TBS to SD (37.72±12.53) than SB (50.18±12.12) ($p=0.01$), while no significant differences were found between GP and PBU (45.16±12.97) ($p=0.136$), PBU and SB ($p=0.483$). On the other hand, all the tested adhesives presented significantly similar bonding to CAD ($p=0.083$).

Conclusions Regardless of the adhesive system, bonding to CAD was lower than bonding to SD. All the tested adhesives showed similar bonding performance to CAD, in etch-and-rinse mode, however bonding to SD was affected by the type of the adhesive.
Enamel Microtensile Bond-Strength Characterization of an Experimental-Adhesive Containing a Dendrimer

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Objectives
To characterize the micro-tensile bond strength (μTBS) to enamel of an experimental Bis-GMA-free adhesive containing a second-generation dendrimer, namely GIEMA.

Methods
Two experimental adhesives systems were formulated, one with Bis-GMA as a bulk monomer (EM1), and an alternative one containing GIEMA as a substitute of Bis-GMA (EM2). For control comparison, two commercial adhesives were chosen: Futurabond® M+ (VOCO) and Scotchbond™ Universal (3M ESPE). Sound human permanent molars were sectioned in halves and randomly assigned to eight groups (n=5): FUT_ER; FUT_SE; SU_ER; SU_SE; EM1_ER; EM1_SE; EM2_ER; EM2_SE, according to the application mode (etch-and-rinse or self-etch). Specimen preparation involved smear layer deposition simulation (600 SiC) and application of the adhesive according to the manufacturer’s instructions. For etch-and-rinse protocol were applied Octacid orthophosphoric acid (37%) (Clarben). Resin build-ups were performed using Schmidt nanohybrid composite (MADESPA). Materials were light-cured with a blue LED light-curing unit, Elipar™ DeepCure-S (3M ESPE) with a peak irradiance 1200 mW/cm². After preparation, specimens were stored in distilled water (37°C for 24 h). Further sectioning was performed to obtain beams (1mm²±0.2) and tested using a universal testing machine (0.5mm/min) until failure. Results were analyzed using linear mixed models (LMM) with fixed effects (significance level of 5%).

Results
Results showed that there was no interaction between the adhesive used and the strategy employed (p=0.985), and therefore, the effects of adhesive (p=0.033) and strategy, or protocol, (p<0.001) on the micro-tensile bond strength are significant and independent of each other.
SBU showed a significantly higher μTBS than experimental EM2 (p=0.031), independently of application protocol.

Conclusions
SBU outperformed the experimental EM2, while the rest of the evaluated UAs were similar in enamel. GIEMA seems to be a favourable alternative monomer to Bis-GMA, with comparable adhesive properties.
O200

Surface Pretreatments/Proximal Box Elevation on Adhesion of CAD/CAM Materials

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Objectives This study aimed to investigate the effect of different surface pretreatment methods on the shear bond strength (SBS) of hybrid ceramic CAD/CAM blocks with/without the use of proximal box elevation (PBE).

Methods Totally 75 cylinder-shaped hybrid ceramic CAD/CAM blocks were prepared (Cerasmart,GC Corp, Japan) (2.4 mm diameter) and randomly divided into 5 groups(n=15).

- Group 1: Sandblasting applied CAD/CAM blocks bonded to dentin surfaces.
- Group 2: Sandblasting applied CAD/CAM blocks bonded to composite surfaces.
- Group 3: Hydrofluoric acid etched CAD/CAM blocks bonded to dentin surfaces.
- Group 4: Hydrofluoric acid etched CAD/CAM blocks bonded to composite surfaces.
- Group 5: Cohesive strength of the CAD/CAM blocks (control)

Mid-coronal dentin surfaces that were obtained from human molars, used in Group 1 and Group 3. Disc-shaped resin composites (Gradia Direct Posterior, GCCorp, Japan) (5 mm diameter) were used in Group 2 and Group 4.

Dual-cure adhesive resin cement (RelayX Ultimate, 3M ESPE, St.Paul, MN, USA) was used for luting in all groups. The SBS was measured using universal test machine (1mm/min). Failure type was evaluated with a stereomicroscope (15X). CAD/CAM block/composite or CAD/CAM block/dentin interface was observed by using Scanning electron microscope(SEM). Data were statistically analyzed with two-way variance analysis and Bonferroni test (p<0.05).

Results Group 1 showed significantly higher SBS than Group 2. Group 5 showed significantly higher SBS than other groups(p<0.05). There were no significant differences in SBS between Group 3 and Group 4(p>0.05).

Regarding the surface pretreatment methods, no significant differences in SBS were obtained between Group 1 and Group 3 and Group 2 and Group 4(p>0.05). Predominant mode of failure was adhesive type for most of the groups.

Conclusions PBE did not differ in bond strength using hydrofluoric acid while this technique led to lower bond strength of hybrid ceramic CAD/CAM restorations, with sandblasting.

O201

Does Composite Repair Time Affect Repair Protocol, Immediate or Delayed?

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Objectives To evaluate and compare the effect of different surface conditioning on the immediate and delayed repair bond strengths of a composite resin.

Methods Composite discs(diameter=6mm) prepared with Ceram.X SphereTEC A1 and placing it in cavities in auto-polymerized polymethylmethacrylate (Auto-PMMA). Delayed repair(DR) group, after aging in distilled water for three days; immediate repair (IMR) group, immediately roughened with a diamond knife-edge bur(40s), washed, dried, each repair group divided into 5 groups(n=7): (1) negative control (C), (2) Single Bond Universal Adhesive (SBU), (3) only Clearfil SE Bond without primer (SEB), (4) Clearfil SE Bond (SEPB), (5) 37% orthophosphoric acid and Clearfil SE Bond without primer(Etch+SEB). Repair filling was performed using the same composite resin and prepared in standard cylindrical form. (diameter:1mm, height=2mm). Shear force was applied to the interface on a universal testing machine. Analyses was performed by one-way ANOVA and Tukey HSD tests. p values less than 0.05 were considered to be statistically significant.

Results The order of bond strength values(Mpa) of the groups from largest to smallest: SBU-Delayed(D)(66,04±33,42), Etch+SEB-D(60,55±16,53), SEPB-D(55,99±26,71), SEPB-Immediate(IM)(40,22 ±10,24), SEB-D(40,18±14,03), SEB-IM(39,97±11,79), SBU-IM(34,31±9,36), control-IM(28,55±22,54), Etch+SEB-IM(27,75±10,06), control-D(19,61±11,91). DR groups had higher values than IMR groups but in groups that using same protocol only Etch+SEB-D had higher values statistically significant than Etch+SEB-IM. In DR, difference between bond strengths control-D with SBU-D, etch+SE-D, SEBP-D was found to be statistically significant. In IMP, difference between groups are not statistically significant.

Conclusions In immediate repair(IMR), using acid-etching before SE Bond without primer reduced the bond strength but in delayed repair(DR) increased. SBU had highest bond strenght values in DR but not in IMR. Applying adhesive material between resin composites in DR group increased the bond strength more than the IMR group.
Composite Cements Containing 10-MDP Bond to Zirconia Without Separate Primer
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Objectives To evaluate the zirconia-to-zirconia micro-tensile bond strength (µTBS) using different composite cements with/without primer.

Methods Initial Zirconia UHT (GC) sticks (1.8x1.8x5.0 mm) were sandblasted with 29-µm alumina sand (Velopex) at 0.2 MPa. Two micro-specimens were bonded together using four cements with/without their respective manufacturer’s primer/adhesive (G-Cem One and G-Multi Primer, GC; Panavia V5 and Panavia SA, and Clearfil Ceramic Primer Plus, all Kuraray Noritake; RelyX Universal and Scotchbond Universal Plus, 3M Oral Care). Each bonded micro-specimen assembly was light-cured for 40 sec using a high-intensity (>1200 mW/cm²) light-curing unit (Smart Lite Pro, Dentsply Sirona). Specimens were trimmed at the bonded interface to a cylindrical hour-glass shape (diameter: 0.9-1.1 mm). After 1-week water storage (37°C), the specimens were either subjected to immediate µTBS testing or aged for 50.000 thermocycles prior to being tested. Specimens that failed prior to testing (PTF) were considered as 0 MPa. Light-microscopy fracture analysis (adhesive interfacial failure, cohesive failure in dentin, cohesive failure in composite, mixed failure) of all specimens was performed, followed by SEM fracture analysis of selected specimens. Data were analyzed using 2-way ANOVA, Tukey, and Mann-Whitney (α=0.05).

Results Statistics revealed significantly varying data for the variable composite cement but not for aging. No significant difference in µTBS with/without primer and aging was recorded for G-Cem One (GC) and Panavia SA (Kuraray Noritake). A significantly higher µTBS was recorded for Panavia V5 (Kuraray Noritake) and RelyX Universal (3M Oral Care) when applied with their respective primer/adhesive. No significant difference in µTBS was recorded between the four cements applied following their best performance. No decrease in µTBS was recorded for any cement upon aging.

Conclusions Adequate bonding to zirconia requires the functional monomer 10-MDP either contained in the composite cement, when a separate 10-MDP primer is no longer needed, or in the separately applied primer/adhesive.
Minor Salivary Glands Ultrasonography in Establishing Sjögren’s Syndrome Prognosis
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Objectives Ultrasonography is a well-established diagnostic technique for the evaluation of salivary glands in patients with suspected primary Sjögren’s Syndrome (pSS). In particular, the Outcome Measures in Rheumatology (OMERACT) scoring system has been reported to improve the sensitivity of pSS diagnostic criteria. Recently, minor salivary glands ultrasonography using 70 MHz frequencies (Ultra-High Frequency Ultrasonography – UHFUS) has been introduced to evaluate the degree of glandular alteration, with encouraging results. The aim of this study was to compare the UHFUS score of minor salivary glands with histology, in order to assess the possibility to stratify the prognosis of pSS patients.

Methods Patients with on onset of xerophthalmia/xerostomia >3 months were enrolled for evaluation. All patients completed pSS diagnostic work-up, which included serology, functionality tests, minor salivary glands UHFUS and surgical biopsy. UHFUS images were scored using the OMERACT scoring system, assigning scores from 0 (normal glandular parenchyma) to 3 (completely altered glandular parenchyma with the presence of hypoechoic areas and glandular fibrosis). The UHFUS score was compared with the number of lymphocytic foci retrieved on histology. Correlation between UHFUS and histology was assessed using the Pearson’s correlation coefficient.

Results Of the 257 patients included in total, 123 (47.8%) were diagnosed with pSS. No differences were found in terms of age or gender distribution. In all the patients with negative FS, a UHFUS score 0 was found. Conversely, a UHFUS score ≥1 was always found in presence of positive FS, and score 3 was always associated to the presence of >3 foci on histology. Significant correlation (p<0.05) was found between UHFUS and histology.

Conclusions These results support the potential role of minor salivary glands UHFUS in the evaluation of patients with suspected pSS, particularly due to the high correspondence with histology. These findings corroborate the adjunctive value of UHFUS evaluation in the diagnosis and risk stratification of pSS patients.

Women Salivary Metabolome in Aging
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Objectives Metabolomic profiling of whole saliva in health, oral and systemic diseases has been studied in the last decades to identify biomarkers. However, the heterogeneity of the results published on breast cancer, oral cancers or Sjögren’s syndrome questions the potential impact of saliva collection procedures and analytical methods used for the identification of potential biomarkers. As Sjögren syndrome is strongly associated with sex (women) and age, the identification of biomarkers of this disease needs a preliminary characterization of the impact of aging on women’s salivary metabolome. Thus, the aims are (i) to compare the metabolomic profile of three salivary sampling processes and (ii) to assess the differences in the metabolite profile with aging using an untargeted approach.

Methods We developed a targeted analytical method using liquid chromatography with both polar and non-polar stationary phases coupled to mass spectrometry used in positive and negative modes (LC/MS) to quantify 82 compounds representative of the wide panel of molecular masses and polarities previously described for salivary metabolites. Then, saliva samples from 18-45 years old women were compared to those of women older than 55. MetaXCMS was used to highlight the statistical differences (based on student t-test) in the expression of metabolites between the two groups.

Results Salivary metabolites obtained after spitting, aspiration and Salivette® were compared. Spitting and aspiration gave statistically similar results for metabolite determination whereas Salivette® gave lower concentrations for most compounds and was not adapted to a global untargeted metabolome analysis. The results demonstrated an alteration of the women’s salivary metabolome (compounds up or down-regulated) in aging.

Conclusions In conclusion, our study showed that the standardization of the whole analytical procedure (saliva collection and LC/MS analysis) is mandatory to allow the proper characterization of metabolites impacted by aging. This analytical procedure could then be applied to identify salivary biomarkers of Sjögren’s syndrome.
Artificial Intelligence for Analysis of Salivary Gland Tumours

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Objectives This study aims to examine if artificial intelligence (AI) can be used to differentiate between different salivary gland tumours (SGT) subtypes based on the analysis of digitised whole slide images (WSIs) of Haematoxylin and Eosin (H&E) stained slides.

Methods A total of 240 scanned H&E WSIs were obtained. An open-source bioimage analysis software (QuPath) was used for training and analysis of features on representative regions-of-interest (ROIs). The first machine learning (ML) classifier was trained and tested to differentiate between two benign and four malignant SGT with an equal split between benign and malignant SGTs (n=120 each). The second ML classifier was used for malignant SGT subtyping (n=120) and results compared with deep learning (DL) methods using multiple state-of-the-art deep learning convolutional neural networks (CNNs). A third ML classifier was used for automated grade prediction in two most common gradable SGT.

Results Our first classifier showed good accuracy for automated differentiation between benign and malignant SGT (F1-score=0.80). The second ML classifier (malignant subtyping) produced a reasonable F1-score of 0.71 for differentiation between four malignant SGT subtypes. Significant differences between cellularity, nuclear haematoxylin, cytoplasmic eosin and nucleus/cell ratio (p<0.05) were seen for both experiments, potentially signifying important diagnostic features. Most DL CNNs achieved higher F1-scores, with ResNet-50 giving F1 scores of 0.86 and 0.69 for benign versus malignant and subtyping respectively. The grading ML classifier also showed good performance (F1-score= 0.73) with significant differences between seen for cellularity, nuclear circularity, eccentricity, cytoplasmic eosin and nucleus/cell ratio (p<0.05).

Conclusions Our novel findings show that AI can be used for automated differentiation between benign and malignant SGT, subtyping and grading on H&E images. Analysis of a larger multicentre cohort using ML and DL at the WSI level is required to improve accuracy and establish the significance of these findings.
Salivary Proteome in Patients With or Without Ulcerative Oral-Mucositis
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Objectives Oral mucositis (OM) is a common debilitating complication of preparative chemotherapy before autologous hematopoietic stem cell transplantation (ASCT). It is clinically characterized by erythema, edema and ulcerations. The aim of this study was to identify differences in the salivary proteome before and after ASCT between patients who developed ulcerative OM (uOM) and those who did not (nOM).

Methods To detect lower abundant proteins, a label free proteomics approach was used with Data Dependent Analysis (DDA) and Data Independent Acquisition (DIA). Stimulated saliva samples from 9 uOM and 10 nOM patients at 6 different timepoints (baseline, 1, 2, 3 weeks and 3 and 12 months after ASCT) were selected from the H-OME study (Dutch extension of Orastem) and analyzed. As spectral library for the DIA analysis, all samples were grouped (uOM vs nOM) and analyzed in DDA mode.

Results The week 1-3 samples clustered differently from the baseline, 3 and 12 months samples. A comparison of the label-free quantification intensities between week 1-3 and the other timepoints revealed that 37 proteins were significantly regulated. During week 1-3, the UP-regulated proteins were mainly involved in keratinization while the DOWN-regulated proteins were mainly involved in the antibacterial humoral immune response. Over all timepoints, 20 proteins were significantly differently regulated between uOM and nOM. The 12 UP-regulated proteins in the nOM group were significantly enriched in immune response related biological processes. Also in the grouped analyses (DDA), the unique and UP-regulated proteins in the nOM group were mainly involved in immune system-related processes. In the uOM group intracellular proteins dominated, most likely reflecting cell lysis.

Conclusions The salivary proteome was indicative of either more protection in nOM and more damage in uOM patients, respectively.
Majority of Hematopoietic Stem Cell Transplantation Recipients Develops Hyposalivation

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Objectives Hematopoietic stem cell transplantation (HSCT) is an established treatment option for many blood diseases. After a conditioning regimen, consisting of chemotherapy with or without total body irradiation (TBI), stem cells from a donor (allogeneic HSCT) or from the patient (autologous HSCT) are infused. A reduced salivary flow rate is a potential complication from HSCT, but the prevalence and duration of, and risk factors associated with hyposalivation are largely unknown. Therefore, we aim to describe the development of hyposalivation in HSCT recipients, and determine risk indicators.

Methods A multi-center prospective observational study (Orastem/H-OME) was conducted in the Netherlands. Unstimulated and stimulated whole saliva was collected before HSCT, during hospitalization, and after 3, 6, 12 and 18 months.

Results 112 HSCT recipients were included. The mean stimulated salivary flow rate decreased dramatically after high intensity conditioning. Changes in flow rates were less pronounced in patients receiving reduced intensity conditioning. During hospitalization, 91% of the autologous and 47% of the allogeneic HSCT recipients was diagnosed with hyposalivation of stimulated saliva (<0.7mL/min), and 70% of the autologous and 40% of the allogeneic HSCT recipients had hyposalivation of unstimulated saliva (<0.2 mL/min). The intensity of the condition regimen was a significant risk indicator for the development of hyposalivation (UWS: OR 5.8, p=0.008; SWS: OR 12.6, p <0.001), while TBI was not. Although mean salivary flow rate increased again after discharge, 12 months post-treatment 25% was still diagnosed with hyposalivation and half of the patients had a lower flow rate in comparison to their own baseline level.

Conclusions Reduced salivary flow rate is a very common complication after HSCT. Even though the reduction tends to diminish over time, in about 50% of patients, both stimulated and unstimulated salivary flow rates are still reduced after 12 months.
Body Mass Index and Caries: Statistical and Machine Learning Analytics

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Objectives To analyse the association between Body Mass Index (BMI) and dental caries.

Methods This research analyzed data from the Dental, Oral, Medical Epidemiological (DOME) records-based study, which integrates comprehensive socio-demographic, medical, and dental databases of a nationwide sample of dental attendees to military dental clinics for 1 year aged 18-50 years. Obesity categories were defined according to the World Health Organization: underweight: BMI < 18.5 kg/m^2, normal weight: BMI 18.5 to 24.9 kg/m^2, overweight: BMI 25 to 29.9 kg/m^2, and obesity: BMI ≥ 30 kg/m^2. General linear models were used with the mean number of decayed teeth as the dependent variable across BMI categories, adjusted for sociodemographics, systemic conditions, and health-related habits. We run XGBoost machine learning algorithm on the same set of clinical features to explore features importance according to the dichotomous target variable of decayed teeth.

Results The study included 66,790 subjects, mean age 22.8±7.1. The mean BMI score was 24.2±4.3 kg/m^2. The distribution of BMI categories: underweight (3113 subjects, 4.7%), normal weight (38924 subjects, 59.2%), overweight (16966, 25.8%), and obesity (6736, 10.2%). Compared to normal weight (2.02±2.79), the number of decayed teeth was statistically significant higher in subjects with obesity [2.40±3.00; OR=1.46 (1.35-1.57)], underweight [2.36±3.04; OR=1.40 (1.26-1.56)] and overweight [2.08±2.76, OR=1.05 (1.01-1.11)]. Following adjustment the associations persisted for obesity [OR=1.56 (1.39-1.76)] and underweight [OR=1.18 (1.004-1.39)], but not for overweight [OR=1.04 (0.96-1.13)]. Important features according the XGBoost model were socio-economic status, teeth brushing, birth country, sweetened beverages consumption, which are well known risk factors of caries. Among those variables was also our main theory independent variable: BMI categories.

Conclusions The study demonstrates an association between BMI and caries, independent of the socio-demographic, systemic conditions and health-related practices that were analyzed. Better allocation of resources is recommended, focusing on populations with underweight and obesity in need of dental care.

Automated Deep-Learning-Based Arrangement of Periapical Radiographs

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Objectives Periapical radiographs are oftentimes taken in series, aiming for establishing an oral status using this imagery. Our aim was to assemble series of periapical radiographs into an anatomically correct status using a deep learning model.

Methods 4,707 periapical images from 387 patients (in mean, 12 per patient) were used. Radiographs had been labeled according to its field of view by trained personnel during image generation. The dataset was split into a training-, validation-, and test set, stratified by patient. In addition to the radiograph the timestamp of image collection was extracted and abstracted. A matrix, containing the normalized timestamps of all images of a patient was constructed, representing the order in which images were taken, providing temporal context information to the deep learning model. Using the image data together with the time sequence data a multi-modal deep learning model consisting of two residual convolutional neural networks (ResNet-152 for image data, ResNet-50 for time data) was trained. Additionally, two unimodal models were trained on image data and temporal data, respectively. A custom scoring technique was used to measure model performance by analyzing correct set assembly.

Results The multi-modal deep learning model outperformed the models using only one data stream. Multi-modal modeling predicted tooth labels with an F1-score, sensitivity and precision of 0.84 and an accuracy of 0.99. 47 out of 77 patient data sets were fully correctly assembled; in the remaining ones, usually only one image was incorrectly labeled.

Conclusions Multi-modal modeling was superior to using only one data stream.
O211
Pilot Online Health Program Improved Mouth Dryness
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Objectives To assess the effectiveness of the Online health program to improve mouth dryness. This abstract focused on the self-perceived psychological health, and xerostomia measures.
Methods In total, 46 participants were included at the baseline, 12 of them finished the two-month program (participation rate 26.1%). At the baseline, the Online health program included one face-to-face oral health visit together with personalized advice on healthy diet, water consumption and physical activity. It was followed by online breathing sessions aiming to improve psychological health, and regular online reminders about healthy behavior. At the baseline, the participants answered sociodemographic questions. Unstimulated salivary flow (USF) rate was measured, Visual Analog Scale (VAS) of xerostomia, Xerostomia Inventory (XI), self-reported depression symptoms scale (HADS questionnaire), and perceived stress scale 10 (PSS-10) were completed at the baseline and after the program. Mann-Whitney U test was used to compare characteristics between those who finished the program and those who did not, and Wilcoxon Signed Rank test was used to compare measures at the baseline and after the program.
Results Those who finished the program had more years of education (18yrs. versus 16yrs., p=0.028), otherwise the groups were not different in age, gender, and residency. After the program, the median values of USF increased from 2.5ml (IQR8.9) to 4.3ml (IQR9.0). The median VAS score among the participants decreased from 5.0 (IQR8) to 3.0 (IQR7), p=0.003. PSS-10 score was reduced from 23 (IQR30) to 19 (IQR24), p=0.007. The marginal statistical significance was shown in reduction of depression symptoms according to HADS questionnaire, where the median value decreased from 5.5 (IQR13) to 2.0 (IQR10), p=0.054.
Conclusions The pilot Online health program was effective to reduce dry mouth symptoms, and to improve self-reported psychological health. Studies with larger number of participants and longer follow-up are needed to assess long-term effectiveness of this program.

O212
Automatic Hyperparameter Tuning and Image Augmentation for Improving Angle Classification
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Objectives The aim of this study was to study the effects of hyperparameter tuning and automatic image augmentation on an Angle classification system that can categorize dental malocclusions into Angle class I, II and III based on digital intraoral photos.
Methods Our dataset consisted of 605 images of Angle class I, 1038 of class II and 408 of class III, all the images having been reviewed by a group of expert orthodontists, who annotated them with the corresponding labels. We trained ResNet architectures for classification for different combinations of learning rate and batch size. For the best combination we compared the performance of models trained with and without automatic augmentation using 10-fold crossvalidation. We used GradCAM to increase explainability, which can provide heat maps containing the salient areas relevant for the classification.
Results The best combination of hyperparameters yielded a model with an accuracy of 0.63, F1-score 0.61, sensitivity 0.59 and specificity 0.80. For the optimal values of learning rate and batch size the model trained with automatic augmentation improved by 5-10 % for all metrics. Misclassifications were most common between the Angle classes I and II. The GradCAM algorithm highlights those areas considered relevant by the dentists, which means that the model learned to identify the relevant areas in the images for determining the Angle class.
Conclusions We proved that the choice of hyperparameters can drastically affect the performance of deep learning models in dentistry, and that using automatic image augmentation can result in further improvements. Our models successfully managed to categorize the malocclusion based on digital intraoral photos.
Towards Privacy-Preserving AI in Dentistry With Federated Learning
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Objectives Deep learning models carry millions of parameters that need to be trained on sufficiently large datasets to reach clinical accuracy. However, data protection and privacy concerns often limit data availability in medicine and, specifically, dentistry. We aimed to introduce federated learning (FL), which builds on exchanging deep learning model parameters from different institutes instead of exchanging their data.

Methods We simulated FL with three clients on an exemplary problem, multi-class tooth segmentation on panoramic images and compared it to centralized learning using a 5-fold cross-validation approach. All models were trained with the same deep learning architecture (U-Net) and training parameters. The centrally located dataset consisted of 2,988 images and was split into train/validation/test (80%/10%/10%). For FL, data splits were equally distributed over three clients.

Results Centralized learning reached dice scores [standard deviation (SD); 95% confidence intervals (CI)] of 0.89 [0.01; 0.87-1.0], 0.91 [0.01; 0.89-1.0] and 0.89 [0.02; 0.87-1.0] for clients 1-3, respectively. FL achieved performances of 0.88 [0.01; 0.86-1.0], 0.89 [0.01; 0.88-1.0], 0.88 [0.01; 0.86-1.0] for the same clients. No significant difference in performance was observed between centralized and federated learning for clients 1-3 with p-values of 0.32, 0.15 and 0.34 (t-test), respectively.

Conclusions FL has the potential to achieve similar results to centralized training while avoiding the exchange of sensitive patient data across centers.

Analysis of YouTube Videos Related to Bichectomy
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Objectives Nowadays, YouTube™ is one of the best sources for people who need to get information about some specific issues. Bichectomy is the surgical procedure that partially removes oral fat. The aim of this study was to evaluate the accuracy and quality of the information content of popular YouTube™ videos about bichectomy.

Methods The keywords “Bichectomy, buccal fat removal” were searched on YouTube™. It was filtered by the view counts and 60 videos that met the inclusion criteria were evaluated. Each video was qualificated with Global Quality Scale (GQS) and for target group. Moreover, videos were scored with Reliable Scale and Content Scale by our authors. Kruskal-Wallis test and chi-square test were performed for statistical analysis. Intra-observer and inter-observer agreement were calculated Cohen’s Kappa index.

Results The videos uploaded by healthcare professionals, GQS(p=0.381) Reliability Scale(p=0.314), Content Scale(p=0.394) were significantly different from the videos uploaded by patients and magazine content. However, there was no significant difference for views(p=0.188), likes(0.068), dislikes(0.297), comments(0.081), duration of the video(0.116) and daily viewing rates(0.114).

Conclusions Assessment of videos described us that videos made by professionals were more qualified for the patients. But videos that made by advertisers or patients have not enough information for people who need to know about bichectomy. YouTube™ can be a platform with the potential for misleading in the bichectomy. Health-related issues should not be tried to be resolved without professional support.
O215
Do Medicated Psychiatric Patients Differ From Other Dry Mouth Patients?
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Objectives In previous studies, we have evaluated oral and ocular health in patients with primary Sjögren’s syndrome (pSS) and patients radiated for head and neck cancer (HNC) demonstrating many similarities between the groups. In the present study, we explored whether medicated psychiatric patients (MPP) have comparable oral and ocular challenges.

We aim to investigate oral and ocular symptoms and clinical findings in MPP suffering from anxiety and/or depression treated with at least one antipsychotic or antidepressive drug.

Methods Twenty-three MPP were recruited during short-term hospitalization. Oral Health Impact Profile 14 (OHIP-14) was used to explore oral health-related quality of life. Subjective oral and ocular dryness were evaluated using Shortened Xerostomia Inventory (SXI) and McMonnies Dry Eye Questionnaire (MDEQ). Oral examination included Clinical Oral Dryness Score (CODS), unstimulated (UWS) and stimulated whole saliva (SWS). Tears were collected using Schirmer test (ST). Results were compared with age and sex matched healthy controls and analyzed using Wilcoxon Rank Sum Test (p<0.05).

Results Subjective significant differences between MPP and controls: OHIP: 17.9±12.8 vs 1.0±1.9, SXI: 10.4±2.7 vs. 6.0±0.7, and MDEQ: 12.5±5.1 vs 3.1±2.6. Clinical significant differences: CODS: 5.1±1.9 vs 0.9±1.9 and SWS: 1.4ml/min±0.8 vs 2.0ml/min±0.8. Clinical non-significant differences: UWS: 0.3ml/min±0.2 vs 0.4ml/min±0.2 and ST: 17.6mm±12.9 vs 22.1mm±10.6.

Conclusions MPP showed higher OHIP values and complaints of oral and ocular dryness than controls, similar to our previous findings in pSS and HNC patients. All patient groups had high CODS, but differed regarding saliva secretion rate; while pSS and HNC patients demonstrated low UWS values, MPP did not. Pathologically reduced tear secretion was demonstrated in pSS patients only. It therefore seems that etiology plays a role in dry mouth patients.


O216
Oral Health of Cameroonian Children: Pilot Study in Rural Areas
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Objectives Access to dental care is a public health issue in Cameroon, given the absence of healthcare system and the low density of dental surgeons (1 per 100,000 inhabitants). It is therefore essential to assess the needs in terms of access to oral care, hygiene materials and education in oral hygiene methods.

Methods A pilot cross-sectional study was conducted in Bamendou, Western Region. The study population included 265 children aged 3-18 years who completed with the help of adults a self-questionnaire about their oral hygiene practices and eating habits. A clinical examination of caries, gingivitis, tartar and oral hygiene was then performed to assess their oral health. The children were separated into 3 age groups for analysis: Group I (3-6 years, n=45), Group II (7-11 years, n=157), Group III (12-18 years, n=63).

Results Among the 265 children (F/M ratio 110/155) the prevalence of caries disease (ICDAS≥2) was 78.5%. It significantly increased according to age groups: 62.2%, 80.9% and 84.1% in Groups I, II and III respectively (p<0.01). 95.1% of the 265 children had never visited a dental surgeon. 6.4% of them did not brush their teeth and 17.7% of them did it less than once a day. 73% of children who brushed their teeth only did it in the morning. While almost all children (97.6%) had a toothbrush, only 72.3% used toothpaste exclusively. 14% used other products such as ash, soap, salt, bicarbonate and 13.6% did not use any brushing product. The prevalence of ICDAS≥2 caries was 77.3% when children used a brushing product and 87.5% when they did not.

Conclusions The high prevalence of caries and the inadequate tooth brushing despite acceptable access to hygiene materials highlight the need for oral hygiene education in this population of rural children.
Dental Caries in Irish Children: a Systematic Review

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Objectives To evaluate all national, regional and county level oral health surveys from 1950 to 2021 conducted in the Republic of Ireland and summarise trends in dental caries prevalence.

Methods The following electronic databases were searched up to 28th October 2021: MEDLINE (OVID), Embase, PubMed, Scopus, Web of Science, Cochrane, Lenus Ireland and Google scholar. For unpublished studies the Dublin Dental University Hospital (DDUH) library database was also searched. A total of 5,066 studies were identified of which 36 studies were included in the review using the PRISMA checklist. Agreement between independent assessors was high (Cohen’s kappa: 0.92). Three were national level studies, eight at health board levels and twenty five were county surveys.

Results Three nationally representative surveys were completed in 1953, 1984 and 2002, with periodic regional and county surveys between these time points. The collated dmft/DMFT scores and the percentage of caries free children for ages 5, 8, 12 and 15 years olds are shown in Tables 1 & 2. The greatest decline in dental caries was between the 1953 and 1984 surveys. This decline was evident for both fluoridated and non-fluoridated areas. The regional and county surveys reflected similar trends and frequency distributions.

Conclusions The reduction in cavitated dentinal caries was most evident between 1953 and 1984 and the declining rate continued from 1984 until 2002 in surveys. Caries levels were lower for children in fluoridated areas for all surveys.

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Mean dmft/DMFT scores in primary and permanent dentitions as reported in the national oral health surveys in the Republic of Ireland. Pre-F = Pre-fluoridation, F = Fluoridated, NF = Non-Fluoridated.

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Mean proportions with caries free primary and permanent dentitions as reported in the national oral health surveys in the Republic of Ireland. Pre-F = Pre-fluoridation, F = Fluoridated, NF = Non-Fluoridated.
GRK5 as a Functional Bridge Between Periodontitis and Neurodegeneration

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Objectives Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by neuronal loss and cognitive decline that often coexists with periodontitis. In this regard, recent studies have demonstrated that Porphyromonas gingivalis (Pg) and its virulence factors (i.e., gingipains and LPS-Pg) have been detected in the brain samples from people with AD supporting the existence of a direct link between periodontitis and AD development/progression. However, the mechanisms connecting these disorders remain still under investigation. In this study, we analyzed the effects of LPS-Pg in neurons and we investigated the role of the G protein-coupled receptor kinase 5 (GRK5), whose deficiency is associated with neurodegeneration development.

Methods SH-SY5Y neuronal cells have been stimulated or not with LPS-Pg (10 ng/mL) for 12 hours. Prior to stimulation, a group of cells was transduced with an Adenovirus (Ad) encoding for GRK5 or GFP as control.

Results Stimulation of SH-SY5Y with LPS-Pg resulted in increased phosphorylation levels of tau (pTau) and beta-amyloid expression. These effects were accompanied by increased levels of the neurogenesis-associated factor GAP43 (Growth-associated protein 43). Next, we observed that LPS-Pg stimulation induced a robust increase in iNOS and 4-HNE levels, suggesting a potential implication for this virulence factor in oxidative stress in neurons. Notably, most of the effects induced by LPS-Pg were abolished in cells overexpressing GRK5 (Ad-GRK5) compared to those expressing GFP as control.

Conclusions The augmented expression of GAP43 is known to be associated with AD and with the increase in amyloid plaque and tau neurofibrillary tangles in AD brains. Therefore, the modulation of the mentioned above molecules by LPS-Pg supports Pg-targeting as a novel strategy to fight AD development and progression. Further, the negative impact of LPS-Pg on GRK5 expression suggests that this kinase plays a pivotal role in preserving neuronal function and survival in patients with periodontitis.
Is the way Researchers are Evaluated Appropriate? a Pilot Study
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Objectives The metrics currently used to evaluate researchers focus on quantity over quality of scientific production and may lead to bad practices due to pressure for publication. The objective of this pilot study was to evaluate a questionnaire for measuring the perception of researchers about research integrity, open science practices, and the researchers' evaluation system.

Methods The online self-administered questionnaire was sent to all 1,296 master’s students attending a Brazilian University. 134 (10.3%) completed the survey.

Results Most of the respondents answered that the questionnaire is comprehensible and easy to respond. A recurrent difficulty reported is that they don’t know exactly how the researchers' evaluation system is. Regarding the perception of the way to evaluate researchers in Brazil, only 10.4% (n=14) of the respondents affirmed that 'the process answers all requirements and must be maintained'. Most of them answered that 'the process answers the most important requirements but may be improved’ (29.9%) or that 'the process needs to be reviewed because it partially answers the most important requirements’ (29.1%). About the assessed activities, most of them reported that they are in general essential or important to advancing career, advancing science, personal satisfaction, and social impact, except for the activity ‘publishing more scientific papers than other researchers’ that was evaluated as important only to the careers advance (p<0.001).

Conclusions The results show that these future researchers realize that the current evaluation system is not ideal, but don’t yet have a clear perception of the differences between activities that promote real advancement of science rather than those that just are used to evaluate researchers numerically. However, the data should be interpreted cautiously because of the very low response rate. The questionnaire proved to be reliable, but a strategy for raising response rates needs to be developed before the application of this tool.

Open Science Practices for Reproducible Data Analysis of Dental Research
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Objectives Adopting reproducible research practices has become increasingly important as a way of contributing to the validity of scientific results. Literate programming embodies the concept of integrating original code and documentation within the same document. This paper describes some of the open science tools for data acquisition and analysis used, and R packages developed, by our research team investigating diet and oral health.

Methods A review of existing internal practices in research data processing was carried out and a checklist developed to establish new training and tools with an emphasis on coding and data analysis skills. A template for a repository structure was created. R packages were developed to simplify data import and renaming of variables. Kobotoolbox and Foodbook24 were selected as tools for collecting field data for oral examination and dietary assessment. Dropbox was used for raw data cloud storage and a data.YAML file created to load the configuration files.

Results Key areas transitioned to were data acquisition-organisation, literate programming and version control with GitHub for collaboration and online publishing. Reorganisation of projects involved multiple approaches including file naming conventions, remapping files and the use of R.Projects configured to sync directly online with GitHub or via the terminal in the RStudio IDE. R Markdown and the knitr package was the favoured tool for reproducible data analysis while internal communication utilised simple markdown language. Literate programming using R Markdown facilitated integration of code, analysis with text and producing manuscripts in latex. Dashboards were created with RFlexdashboard and interactivity added with the shiny app which was shared using the open source Github pages.

Conclusions Transitioning to reproducible research involves adopting tools and multiple tasks that can improve efficiency, collaboration and increase transparency but requires investment in training and data science skills.
Automatic Caries Detection in Bitewing Radiographs Using Convolutional Neural Networks
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Objectives Bitewing radiographs are periodically taken during dental check-ups, primarily to detect interproximal caries. However, the analysis is subjective, and clinicians may fail to notice some carious lesions, especially under time pressure. Therefore, this study aimed to automate caries detection in bitewing radiographs using convolutional neural networks (CNNs).

Methods After obtaining approval of the institutional ethics committee, 3500 anonymized bitewing radiographs (1068x847 pixels) were exported from the university hospital database. In total, 6087 carious lesions were annotated using the Computer Vision Annotation Tool. The dataset was divided into 3 parts for the training (70 %), testing (15 %) and validation (15 %). Several CNN architectures were tested to optimize the outcome. Caries detection was considered positive if the intersection over union of the prediction and respective annotation was greater than 0.5.

Results The average precision (AP50) calculated as the area under the precision-recall curve was 69.4 %. When divided according to lesion size, the lowest AP of 60.5 % was achieved for small caries (<32x32 pixels), followed by 72.9 % for medium caries and 80.3 % for large caries (>96x96 pixels).

Conclusions CNNs provided promising results, but a larger training dataset will be required to increase the accuracy of predictions, especially for small carious lesions. On the other hand, the review of annotations and predictions revealed that even the current model successfully detected some carious lesions that were overlooked by the annotators.

Preparation and Characterization of a Radiopaque Infiltrant for Early Enamel Carious Lesions
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Objectives An infiltrant for early enamel carious lesion should have a radiopacity ≥ enamel. The aim of the present project was to prepare a polybrominated monomer that was mixed with an experimental resin infiltrant, at different percentages, to attain a radiopaque resin infiltrant.

Methods Tribromoethyl methacrylate (TBrM) was prepared and characterized by 1H and 13C NMR spectroscopy. An experimental infiltrant (EI) composed of triethylene-glycol dimethacrylate (80%) + urethane dimethacrylate (20%) was mixed with TBrM (30%, 50% and 70% wt respectively). Icon® was used as the control sample. The mixed resins were poured into a rubber base mould and light polymerized to fabricate uniform disk. The quality of the polymer network was assessed by measuring the degree of conversion (DC) using Fourier Transform Infrared spectrophotometry (FTIR). Each disc was imaged using an X-ray machine with an aluminium step wedge as a reference. All the images were analyzed using Image J™ software to calculate the mean grey value of each disk, which was then converted to aluminium thickness with equivalent radiopacity.

Results The DC for Icon®, EI, EI with 30, 50 and 70% TBrM were 74.4%, 74.6%, 86.7%, 86.2% and 86.5% respectively. There was no significant differences between the prepared EI and the Icon®, whereas there were significant differences between the brominated EI and Icon®. Moreover, the DC differences among the various loadings of TBrM were not significant. The Al equivalent radiopacity thickness for Icon®, EI, EI with 30, 50 and 70% TBrM were 0.19, 0.25, 2.1, 3.4 and 4.4 mm respectively, showing the radiopacity of EI with TBrM was higher than that of enamel (2 mm Al thickness) (Figure 1).

Conclusions The tribromoethyl methacrylate can endow resin infiltrant with the required level of contrast for detection. Its improved DC indicates that the prepared resins may have satisfactory mechanical properties to support early enamel carious lesions after the infiltration.
Investigation of Effects Produced by Fluoride Releasing Glass Ionomer Cements

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Objectives Following research study is centred around investigating chemical interaction of fluoride releasing Glass Ionomer Cements (GIC) (Fuji IX, Chemfil Superior containing G338 glass, LG26) on surrounding hydroxyapatite (HA) when immersed in solutions like artificial saliva (pH 7) and acetate buffer (pH 4.5). Additionally, it also aims at comparing effects produced on hydroxyapatite discs due to fluoride releasing glasses with non-fluoride releasing glass (LG30) when subjected to varying pH environment. Research includes X-ray microtomographic (XMT) investigation of drilled and subsequently filled (Fuji IX, G338, LG26, LG30) HA discs when immersed in solutions with neutral pH (artificial saliva, pH 7) and acidic pH (acetate buffer, pH 4.5). Also, in order to provide further justification to effects observed in the scans, elemental analytical techniques like Ion Selective Electrode (ISE) and Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) were utilized.

Methods 12mm by 2mm HA discs constituting 20% porosity were drilled to 2mm with tungsten carbide bur. Holes were then restored with GIC that was prepared on a cool glass slab. The filled disc was further incubated for 1 hr in 100\% relative humidity to allow for complete setting of the GIC. Finally, the discs were immersed in 10ml of solution. Elemental analysis was done at 1hr, 9hrs, 24hrs, 1 week, 1 month and 3 months from time of immersion. The discs were scanned after 3 months of immersion.

Results Discs that were filled with fluoride releasing GICs showed a thin radiopaque “ring” around the restoration margins after immersion in artificial saliva. This effect was more exacerbated under acidic pH. Conclusions It can be linked to release of F\textsuperscript{-} accompanied by uptake of OH\textsuperscript{-} into restoration thus resulting in decreased pH causing subsequent dissociation of Ca\textsuperscript{2+} and PO\textsubscript{4}\textsuperscript{3-} ions from apatite and reprecipitation near the margins.
O226
S. Mutans Biofilm Removal by α-(1→3) Glucanase and DNase
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1Biosciences, UNICAMP, Piracicaba, Sao Paulo, Brazil, 2Pólo TerRA, USP, Sao Carlos, Sao Paulo, Brazil

**Objectives**
Extracellular polysaccharides (EPS) are present in the matrix of oral biofilms, contributing to bacterial accumulation and protection against antimicrobial agents. Moreover, eDNA may favor EPS stabilization. Therefore, the degradation of EPS by α-(1→3) glucanases (GH87) and eDNA by DNAses could be a strategy for biofilm control, also reducing the need to use antimicrobials. Therefore, we conducted a pilot study to evaluate the enzymatic activity of α-(1→3) glucanase (GH87) and DNAse in Streptococcus mutans biofilm removal.

**Methods**
S. mutans UA159 biofilms were formed for 24 h in 96-well plates using UTEYB medium supplemented with 1% sucrose. The biofilms were submitted to an enzymatic treatment (0.5 mg/ml in PBS for each enzyme) at 37 °C for 1 h according to the following groups: DNAse, GH87, DNAse + GH87 and PBS (negative control). Then, biomass was stained with 0.05% crystal violet, water washed and the remaining dye was recovered with 30% acetic acid, being measured in a microplate reader at 570 nm. The % of biomass reduction was calculated for each treatment group in relation to the control. Data were analyzed by one-way Anova, Tukey’s Test (α=5%).

**Results**
The % of biomass reduction (mean ± SD; n=6; different letters represent statistically significant differences, p<0.05) for DNAse, GH87 and DNAse + GH87 were, respectively, 11.1 ± 7.7A, 33.7 ± 20.9B and 80.6 ± 3.6C. The % of biomass reduction was higher when the enzymes were combined (80.6 %) compared to the sum of their isolated effects (44.8 %).

**Conclusions**
In conclusion, the data suggest that α-(1→3) glucanase and DNAse combined have a synergistic effect on the removal of S. mutans biofilm.

O227
Vimentin Mediated Downregulation of Intercellular Associations Augments Cancer Cell Migration
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**Objectives**
Vimentin protein expression is induced in epithelial cells during EMT and cancer metastasis. In this study we have investigated the effect of vimentin expression on the transcriptome of human breast cancer cell line, MCF-7.

**Methods**
To recapitulate the events during EMT, full length vimentin was sub-cloned in pLPC hygro vector and transduced in MCF-7 to make MCF-7 FV, stably expressing vimentin, and MCF-7 CV as control vector. Vimentin was knockdown by using VIM sh-RNA in MCF-7 FV and MDA-MB-231 to make MCF-7 sh-Vim MDAMB-231 sh-Vim cell lines, respectively. Cell proliferations assays, colony assay, migration assay, morphological assay, qPCR and RNA-seq analysis were performed to investigate the effect of vimentin in MCF-7 cells.

**Results**
Our results indicated that vimentin expression in MCF-7 cells induced cell migration significantly while its knockdown reverses the effect on cell migration. Cell proliferation rate was not affected. RNA-seq analysis showed that a total of 598 genes were differentially expressed (DEGs), 282 (47.16%) genes were downregulated and 316 (52.8%) genes were upregulated in MCF-7 FV as compared to MCF-7 CV. RNA seq data was validated by qPCR. Functional analysis showed that vimentin mostly affect the ECM related genes and their interactions. We analysed 30 DEGs and were able to find out a common gene signature affected by vimentin in MCF-7 sh-Vim and MDA-MB-231 sh-Vim cell lines that was reverse in MCF-7 FV implying a possible functional link between vimentin and these genes. These include WISP2, FGF4, LINCO00052, KIF26A, NES, CDC20, BCL2, FSD1, E2F1, CDH10, CD109, TFP12, TGFβ1, CEACAM1, CDH5.

**Conclusions** Our data suggest that most of the genes affected by vimentin are linked to ECM and cell-cell junctions. The downregulation of genes linked to cell-cell junctions indicates that vimentin induced cell migration involves suppression of intercellular associations thus making cells freer to move faster.
Investigation of Oral Cancer Microenvironment by Synchrotron Phase Contrast μ-CT

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Objectives The study analysed the 3D organization of the tumor stroma of oral tongue squamous cell carcinoma (OTSCC) by Synchrotron Phase Contrast μ-CT to evaluate the tumor microenvironment (TME) rearrangements.

Methods Thirty-five primary OTSCC were selected from the archive of Marche Polytechnic University, Italy, and stratified according to the pathological staging. For each case, a paraffin-embedded tissue block was selected, carried out from the most invasive part of the tumor. A cylindrical section (<2mm/side) was collected from each block, by selecting the invasive area with the highest percentage of desmoplastic stroma. The high resolution μ-CT was performed at the SYRMEP beamline, ELETTRA Synchrotron Facility, Italy. The collagen specific volume (CollV), the anisotropy degree (DA), the connectivity density (ConnD), and the fractal dimension (FD) of peritumoral stroma were evaluated by the DragonFly 2022.1 Software and the “Frangi 3D” filter. For each case, several clinicopathological characteristics were retrieved, including tumor stroma ratio (TSR), worst-pattern of invasion (W-POI), main POI (POI-max), and the immune-phenotypes.

Results Results showed multiple significant relationships between different parameters in OTSCC tumor stroma, in particular between DA and CollV (p=0.005), DA and FD (p=0.014), CollV and FD (p<0.0001), ConnD and FD (p=0.001), and ConnD and CollV (p<0.0001). Regarding clinicopathological features, FD and CollV values proportionally increase with the amount of the TSR (p=0.036 and p=0.008, respectively). ConnD value was higher in OTSCCs with greater W-POI and POI-max (p=0.002 and p=0.011, respectively). Furthermore, ConnD in OTSCCs with immune-excluded and immune-desert phenotypes was higher compared to inflamed immune-phenotype (p=0.024).

Conclusions These data suggest an association between lower TSR and higher collagen density and tumor 3D irregularity and complexity. Furthermore, a higher ConnD was found in healthy stroma compared to tumor stroma. The Synchrotron Phase Contrast μ-CT allows to reliably discriminate between healthy and pathological collagen distribution, contributing to understand the TME rearrangements during OTSCC cancerization.
Tumor-Associated Collagen Signatures by Multiphoton Microscopy in Oral Cancer.
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Objectives
During malignant transformation, remodeling of the extracellular matrix creates a stromal tumor microenvironment (TME) essential for cancer progression. Collagen is a main component of this TME and its relevance as an indicator of tumor transformation is highlighted in research on breast and prostate cancer. The most sensitive reported technology to study these collagenic architectural transformations is multiphoton microscopy (MPM), monitoring 2-photon excited fluorescence and second harmonic generation. The main objective of this work is to establish a proof of concept of MPM to better understand the TME processes underlying oral cancer (OC) and to support novel effective therapeutic strategies. The relevance of the topic gains its evidence from the current lack of effective prevention and curative treatment for this devastating cancer.

Methods
Cryostat-sectioned and paraffin-embedded samples were analyzed and the generated SHG images were recorded using a custom-made MPM based on a Tsunami laser and an upright SliceScope microscope furnished with a multiphoton galvanometer scan head. 38 samples, pertaining to 15 types of lesions representative of all tumor stages, were analyzed. 44% of samples (n=168) were usable.

Results
Results showed that collagen, normally well organized, deteriorates with tumour progression and gradually transforms into an increasingly disordered network, with fibers firstly dissociating and thinning. Analysis by vectorization of perilesimal collagen fibers suggests, in invasive tumors, an organization of these fibers perpendicularly to the tumor, creating "gateways" for migration into surrounding tissues of tumor cells. The identification of the evolution of collagen density and organization seems to allow, as for other tumor localizations, to define a tumor-associated collagen signatures (TACS) gradation scale for OC, statistically correlated with overall and recurrence-free survival.

Conclusions
MPM could be a reliable tool in the identification of tumor progression markers of OC, which would be complementary to pathological examination and useful for identifying target areas for biopsy or surgical margins.
Alcohol Consumption may Enhance Oral Carcinogenesis by Modulating Aldehyde Dehydrogenases.

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Objectives Oral carcinogenesis (OC) is a poorly understood process. Alcohol consumption is a known risk factor for OC but the exact mechanisms by which it exerts its effects are unclear. Aldehyde dehydrogenases (ALDH) are a family of ethanol-metabolising enzymes involved in detoxification, biosynthesis, proliferation, differentiation, and other cellular activities. Utilising gingival and buccal squamous cell carcinoma and dysplastic cell lines, the objectives were to determine the effect of chronic alcohol consumption on the expression/activity of ALDH isoforms, the transformation of dysplastic cells and on the metastatic characteristics of cancer cell lines.

Methods Cells were treated with ethanol (1% v/v) ‘chronically’ (2 wk) or ‘acutely’ (24 h). ALDH activity/expression was determined by enzyme assay, RT-PCR, immunofluorescence and flow cytometry. Cell migratory and invasive capacities were tested via anchorage-independent growth and scratch assays.

Results The oral cell lines possess unique expression profiles of ALDH isoforms. Only dysplastic DOK cells possess the ALDH1A1 isoform associated with retinol metabolism, drug resistance and worse overall prognosis for OC. Chronic alcohol exposure decreased overall ALDH activity/protein expression in all cell lines, increased cell migratory behaviour in gingival carcinoma Ca9.22 cells, and increased capacity for anchorage-independent growth in DOK cells - a marker of carcinogenic transformation. These effects were not attributable to equivalent acetaldehyde concentrations.

Conclusions By affecting the expression/activity of ALDH in oral cell lines, chronic alcohol exposure decreases cells capacity for detoxification and clearance of harmful acetaldehyde. Simultaneously, chronic alcohol modulated cellular activities that are characteristic of malignant transformation and metastasis; promoting cell growth and migration. This research suggests that alcohol consumption can contribute to these processes via ALDH modulation, making it a potential therapeutic target. Similarly, ALDH expression profiles of patients may be used as a useful biomarker to identify those at higher risk for oral carcinogenesis.

Proteomic Comparison of Pleomorphic Adenoma and Carcinoma Ex-Pleomorphic Adenoma

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Objectives The aim of this study was to compare the proteome of normal salivary gland (NSG) (n=6), pleomorphic adenoma (PA) (n=7) and carcinoma ex-pleomorphic adenoma (CAExPA) (n=7).

Methods Liquid chromatography-tandem mass spectrometry was performed on normal salivary glands and benign and malignant tumour-enriched samples. Protein-protein interaction networks, KEGG enriched pathways and gene ontology analyses of differentially abundant proteins were carried out and Perseus software used for statistical analysis.

Results Differential protein expression between NSG, and PA identified 35 up-regulated and 28 down-regulated proteins, while 31 proteins were up-regulated and 17 down-regulated in CAExPA. Decorin, a member of the small leucine-rich proteoglycans found in the extracellular matrix (ECM), was the most up-regulated protein in both tumours compared to NSG (PA and CAExPA log2 fold change of 7.58 and 7.38, respectively). Biglycan, which is also proteoglycan, was the most down-regulated protein in neoplastic samples compared to NSG (PA and CAExPA log2 fold change of -9.96 and -7.29, respectively). PA and CAExPA also differed in their proteome. Twenty-one proteins were up-regulated in PA compared to CAExPA, with Protein transport protein Sec24A demonstrating the greatest difference (log2 fold change of 4.12). On the contrary, 18 proteins were up-regulated in CAExPA compared to PA, with the greatest changes being in the expression of Translocation protein SEC63 homolog (log2 fold change 4.40) and Annexin A6 (4.07). Interestingly, Biglycan, which was over-expressed in NSG compared to both benign and malignant tumors, was found to be significantly up-regulated in CAExPA compared to PA (log2 fold change 2.66).

Conclusions Our results suggest that proteins from the ECM play an important role during tumorigenesis of PA and CAExPA. Moreover, Biglycan seems to have a dual role, and could be associated with the pro-tumorigenic profile of CAExPA.
O232
Treatments’ Outcomes in Pemphigus Vulgaris Patients: a Long-Term Comparative Study
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Objectives Pemphigus Vulgaris (PV) is a severe autoimmune blistering disease characterized by bullous lesions initially involving the oral mucosa, then spreading to the skin. Up to now, few studies have investigated the prognosis in relation to the clinical phenotype. Therefore, we aimed to evaluate the relationship between clinical remission and therapeutic regimens comparing subsets of exclusive oral/mucosal (OM-PV) vs mucocutaneous PV (MC-PV).

Methods A retrospective single-center study was conducted, and sociodemographic data, phenotype, prescribed therapies, and related outcomes of PV patients diagnosed between 1994 and 2020 were collected. Descriptive statistics and the Fisher’s exact test were computed.

Results 125 PV patients, 52 OM-PV and 73 MC-PV with a mean follow-up 8.4 ± 5.8 years, were included. The majority of these patients, 74 (59.2%), were successfully managed with conventional immunosuppressive therapy (CIST) alone. However, 51 (40.8%) patients required additional biological treatments (Rituximab +/- IVig), either because non-responders or developed severe side effects from CIST, or because of the rapid and severe progression of the disease. Specifically, there was a statistically significant difference in the distribution of therapeutic regimens between OM-PV and MC-PV, as the 50.7% of the latter needed further biological treatments (p-value:0.008). Nonetheless, the frequency distribution of the complete clinical remission (CCR) was not dependent on clinical phenotype, nor on the type of treatments in both groups (p-values: 0.907, 0.982, 0.763). Overall, 119 (95.2%) of the PV patients achieved CCR, although MC-PV experienced more CIST-related side effects compared to the OM-PV (p-values: 0.01) and adverse events related to the biological therapies.

Conclusions PV treatment may be challenging especially in MC-PV patients, as they often require further treatments in order to achieve the CCR. These results advice for an early diagnosis of the oral/mucosal bullous lesions in order to improve the prognosis.

O233
Development of a Multi-Cellular Tissue-Engineered Model of Oral Lichen Planus (OLP)
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Objectives Despite advances in OLP research, investigations into the condition are hampered by the lack of effective experimental models. Therefore, this project aims to develop tissue-engineered oral mucosal equivalents (OME) containing polarised T-cells to replicate OLP for use in the development of novel treatments.

Methods CD4+ and CD8+ T-cells were isolated fromuffy coats then activated with CD3/CD28 Dynabeads. T-cells were cultured in polarising conditions to polarise CD4+T-cell into Th1 and CD8+T-cells into cytotoxic T-cells. Full-thickness OME was created by seeding immortalised oral keratinocytes (FNB6) on top of normal oral fibroblast-containing hydrogels then OME was activated with IFN-γ and TNF-α. Next, T cells were incorporated in hydrogel and their viability was assessed with PrestoBlue assay. Finally, a model of T-cell mediated inflammatory oral mucosal disease was fabricated in vitro by combining OME on top of a T-cell containing hydrogel and then stimulated.

Results CD4+ and CD8+ T-cells were isolated with high purity (85-95%) and viability (>90%). Upon activation, CD69 levels were increased. Only Th1 cells secreted IFN-γ and TNF-α and showed a higher expression of T-bet. 92% of CD8+ T-cells produced granzyme B. H&E-staining of OME revealed a multi-layered stratified squamous epithelium on top of a fibroblast-populated connective tissue and chemokines relevant to T-cell chemotaxis in OLP were secreted from activated OME. T-cells incorporated within a collagen hydrogel maintained 97% viability. Histological staining of the disease model revealed basement membrane destruction and basal cell-layer apoptosis when compared to control models. Following stimulation, CD4+CD8+T-cells were attracted toward areas of the epithelial damage as in OLP.

Conclusions A basic T cell-mediated inflammatory disease model can be fabricated in vitro and it has reflected morphology, compositions, and markers similar to that found in OLP.
Effervescence: an Innovation in Porous Hydrogel Preparation for Bone Regeneration

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Objectives A major challenge in bone regeneration is the use of scaffolds allowing the provision of oxygen supply to avoid hypoxia. The effervescence is a gas-foaming technique recently used to prepare injectable hydrogels. The aim of this work was to evaluate an effervesce-produced hydrogel as a support for bone regeneration.

Methods Polyethylene glycol and lysine dendrimers were mixed to prepare an injectable hydrogel to be delivered by a dual-chamber syringe and used as a control. Glacial acetic acid and potassium carbonate were added to generate CO2 bubbles within the hydrogel. The resulting porosity was checked for interconnection under optical and fluorescence microscopy and using China ink diffusion. The hydrogel toxicity was evaluated on human Umbilical Vein Endothelial Cells (HUVEC) and Periostium-Derived Cells (hPDC) using the MTT test. The colonization was evaluated by seeding HUVEC and hPDCs on a 3 mm-thick hydrogel disc for 10 days and fluorescence microscopy was used to check their localization within the material (DAPI).

Results Immediately after injecting the hydrogel, numerous spherical structures were visible to the naked eye and were associated with a large volume expansion. These correlated to the newly-formed CO2 bubbles and appeared clearly under the microscope within the hydrogel. The resulting porosity (diameter = 100±30µm) was interconnected as China ink diffused through the full 2 cm-thick cylinder within 2±0.3 min, while no diffusion was observed in the non-porous hydrogel. The MTT test showed that the hydrogel was not toxic. In addition, after plating HUVEC and hPDCs on the hydrogel discs’ surface, DAPI-stained nuclei were visible inside the hydrogel pores at 10 days, indicating the hydrogel colonization.

Conclusions Creating the porosity within the injectable material is innovative and holds promise in providing pathways for cell colonization and vascularization within injectable hydrogels. This may represent an added value in critical size bone defects regeneration.

Apatite Formation Under Extreme Acidic Conditions Using Solely Inorganic Approaches

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Objectives Erosive tooth wear is the loss of dental hard tissues including enamel and dentine, through non-bacterial acid dissolution from intrinsic and/or extrinsic sources. This study aims to challenge conventional wisdom of how to repair eroded enamel surfaces by studying apatite crystal growth at extreme acidic pH conditions mediated by calcium phosphate supersaturated solutions.

Methods Hydroxyapatite powder (100 mM) and sodium fluoride (2mM) was added to 100 ml of deionized water with continuous stirring. Subsequently, 69% nitric acid was added dropwise into the solution slowly until the powder was completely dissolved. Ammonium hydroxide solution (30%) was added dropwise until the pH was readjusted to 2.0. Glass slides were inserted into the solution to evaluate apatite crystals growth and morphology in the first 24 hours through 10 timepoints: 10min, 20min, 30min, 40min, 50min, 60min, 2h, 3h, 4h and 24h. Qualitative analysis of the crystal growth and morphology was conducted using digital polarised light microscope and scanning electron microscope (SEM). Quantitative analysis was performed by using Fourier-transform infrared spectroscopy (FTIR).

Results At pH2.0, apatite seeds were observed in the first 10min. After 1h, the substrate became fully covered with apatite seeds. Flower-like crystals with shuttle-shaped petals could be seen in the first hour with about 500nm length. Crystals morphology remained similar for up to 4h, although the diameter of the structures decreased significantly to about 20nm length after 2h. Some apatite seeds were visualised even after 24h. FTIR analyses revealed a characteristic phosphate absorption band at 1000cm-1, which is typically found in apatite. This peak was present for all timepoints evaluated. No significant variations related to peak shape and intensity were observed among samples.

Conclusions With sufficient availability of calcium and phosphate, apatite crystal growth can occur even under extreme pH conditions in a dynamic fashion.
O238

Morphological and Chemical Analysis of Dentin Affected by Radiation-Related Caries

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Objectives This study aimed to analyse the morphology and chemical composition of dentin affected by radiation-related caries compared to adjacent irradiated dentin and sound dentin.

Methods Six teeth from patients submitted to radiotherapy were used to obtain fragments of radiation-related carious dentin (RRC) and adjacent irradiated dentin (AID) from the same tooth. Fragments of dentin from six sound teeth were used as a control group (SD). The fragments were embedded in resin, polished, dehydrated, and fixed for morphological and chemical analysis by scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS). Other fragments were sectioned, included, and polished for X-ray diffraction analysis (XRD) and Fourier-transformed infrared spectroscopy (FTIR). Data were statistically analysed by Kruskal-Wallis, and the Pairwise Method (p<0.05).

Results A morphological disorganization without discrimination of inter- and peritubular dentin were observed in RRC samples compared with a more homogeneous structure in SD. The chemical analysis showed higher C amounts in RRC than in SD (p = 0.027). The detected amounts of Ca and P were higher in SD than in RRC (p = 0.027 and p = 0.039). The microstructure analysis showed similarities between SD and AID in XRD. FTIR results presented higher phosphate and carbonate bands intensities for the spectrum corresponding to SD, followed by AID and RRC.

Conclusions Adjacent irradiated dentin and radiation-related carious dentin showed obliteration, cracks, and craters. Additionally, chemical degradation as Ca and P %wt decrease and different intensities of the mineral content in radiation-related carious dentin and adjacent irradiated dentin were observed when compared to the control group.

O239

Human Dentine Tubule and Microbranch Variations With Quantitative Serial-Block-Face Scanning-Electron-Microscopy

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Objectives Microbranches (approximate diameter: 0.2-1.0μm) exist within dentine, providing a network of fluid-filled connections between the tubules. This study aims to use Serial-Block-Face Scanning-Electron-Microscopy (SBF SEM) to generate 3D volumes of dentine from different locations within the tooth, to allow for the investigation of the microbranches morphology, the microbranch network characteristics, and how this varies around the tooth.

Methods 3 human teeth were sectioned to generate 21 dentine samples extracted from 7 identical regions-of-interest (ROIs) from around the tooth. SBF SEM was performed using a Gatan 3View, providing a pixel size of 10nm, and a field-of-view of 20.48μm x 20.48μm. Tubules and Microbranches were automatically segmented using Fiji and ORS Dragonfly, with quantification done manually and with Fiji. Quantified parameters included: tubule diameter, density, and orientation; microbranch diameter and density.

Results The microbranches were successfully captured in sufficient resolution to generate 3D images. The microbranches move tortuously through the intertubular dentine and travel a variety of distances before connecting to other tubules, with both tortuosity and distance traveled being independent of ROI location. The microbranch density varied significantly, with none observed nearest the PC, and large amounts found under the cusp and occlusal fissure. Microbranch diameter did not vary significantly, with most approximately 150-250nm, regardless of ROI location. Tubule density and diameter varied radially – largest closest to the pulp cavity (PC), then decreasing to the dentinoenamel/dentinocemental junction (DEJ or DCJ). Tubule orientation was always angled towards the DEJ/DCJ.

Conclusions This study provides new insight into the morphology and network of dentinal microbranches, demonstrating their distance of travel within the tissue but also a variability in the density of the microbranches with location around the tooth.
Identifying Internal Changes in the Teeth in Response to Enamel Cracks Using X-ray Microtomography

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Objectives The aim of the project is
1) To study the defense mechanism in teeth using X-ray Microtomography which is sensitive to detect any mineral changes
2) Scanning electron microscopy (EDX-SEM), and synchrotron X-ray diffraction (SXRD) will also be used to study the reactive mineral responses.

Methods Extracted tooth samples were collected from different age groups (with ethical approval), many showing evidence of having been subjected to a variety of dental insults prior to extraction. These were scanned with high contrast X-ray microtomography (XMT or micro-CT) to identify paths of hypermineralization within the dentine associated with enamel cracks. SEM and synchrotron X-ray diffraction were used to determine the nature of the mineral formed within the crack. Although the mechanism for reactive and reparative dentine formation is understood, preliminary studies have suggested that mineral transport through the dentine tubules may also have a protective effect.

Results Hypermineralisation paths from the pulp to the enamel-dentine junction associated with enamel cracks could be seen in the XMT images. Within these cracks, SXRD exhibited speckle patterns indicating the presence of discrete crystals with sizes of around 2 to 3 microns. Preliminary analysis of the speckle pattern suggests that these are whitlockite crystals, distributed approximately uniformly throughout the depth of the crack. SXRD of calculus formed on the enamel surface and within a fissure (from a different sample) did not show the same pattern.

Conclusions The results suggest that whitlockite was formed as a result of dentinal fluid flowing through the crack. In future experiments, we will identify teeth with both enamel cracks (with evidence of associated dentine hypermineralisation) and enamel calculus on the same sample.
Objectives Periodontitis (PD) is the most prevalent chronic inflammatory disease, characterized by the destruction of the supporting structures of the teeth. The presence of certain bacteria in the dental plaque and the inflammatory host response both play a crucial role in PD’s emergence. As chronic inflammation is able to impact the host immune response locally and systematically, evidence is supporting the existence of a bi-directional relationship between type 2 diabetes (T2D) and PD. However, the pathobiology behind this correlation is not fully clear yet.

Methods 14 Collaborative Cross (CC) mice lines were generated and maintained on a rodent chow diet (CHD). At the age of 8 weeks, 12 mice from each CC-line were maintained on a high fat diet (HFD) and 12 mice were kept on a CHD. The same protocol was applied on all 14 CC-lines to determine heritability and genetic variation of bone volume in a diet-dependent manner. Body weight changes were assessed and glucose tolerance was measured using an intraperitoneal glucose tolerance test. At 20 weeks, the mice were sacrificed and their maxillae were harvested and analyzed for alveolar bone loss (ABL) using microcomputed tomography. Subsequently, 10 pro-inflammatory cytokines from blood serums collected from 6 CC-lines were quantified using multiplex immunoassays.

Results The CC mice have demonstrated power for studying the genetic background impact between comorbidity of T2DM and PD. Micro-CT analysis, IPGTT and bone loss assessments emphasized the impact of genetic variation as all 14 lines reacted differently to the HFD. Some lines were resistant to a HFD-induced ABL, some were susceptible and some showed bone formation. In addition, preliminary results of multiplex immunoassays have showed correlation between pro-inflammatory cytokines levels to ABL.

Conclusions The results of this study showed that a HFD is potentially an inducer of ABL and glucose intolerance, depending on the genetic background of the CC-mice. Through the use of our murine model that mimics the complex genetic diversity among populations, we aim to better understand the pathobiology and to identify the phenotypic and genotypic responses of PD and T2DM separately and in comorbidity.
SARS-CoV-2 Infection Causes Periodontal Fibrotic Pathogenesis Through Deregulating Mitochondrial Beta-Oxidation

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Objectives The global high prevalence of COVID-19 is a major challenge for dental professionals and patients. SARS-CoV-2 virus mutate predominantly in the spike proteins, whilst the other key viral components remain stable. Previous studies have shown that human oral cavity can potentially act as reservoir of the SARS-CoV-2 virus and COVID-19 is likely to be connected with poor periodontal health. However, the consequence of SARS-CoV-2 viral infection on human oral health has not been systematically examined. This study aimed to study the pathological functions of SARS-CoV-2 viral components on human periodontal tissue and cells.

Methods Tissue arrays and 3D equivalents were used for evaluating the presence of the SARS-CoV-2 receptors ACE2 and TMPRSS2 in human periodontal tissues. Viral envelope, membrane, nucleocapsid and spike proteins, either as pseudo virus or recombinant proteins, were applied to test their function on human periodontal ligament fibroblasts, for acute (6 hours) and chronic (48 hours) effects. BrdU incorporation assays were used to assess proliferation, TUNEL assays for apoptosis, and immunohistochemistry for senescence. Collagen I, Smooth Muscle Actin and Matrix metalloproteinase expression were evaluated using immunostaining and RT-PCR. The molecular changes were screened using proteomic analysis and validated with Western Blotting. Mitochondrial function was analyzed using a Seahorse Bioanalyzer, together with pathway specific inhibitor treatment followed by immunohistochemical analysis.

Results Human periodontal tissues, particularly the fibroblasts highly expressed ACE2 and TMPRSS2. Exposure to SARS-CoV-2, especially by the viral envelope and membrane proteins induced fibrotic pathogenic phenotypes, including periodontal fibroblast hyperproliferation, concomitant with increased apoptosis and senescence. The fibrotic degeneration was mediated by a down-regulation of mitochondrial beta-oxidation. Chemical inhibitor treatment could mirror the same pathological consequence on the fibroblasts, similar to SARS-CoV-2 infection.

Conclusions The results provide novel mechanistic insights into how SARS-CoV-2 infection can affect human periodontal health at the cell and molecular level.
P. Gingivalis Promotes the Secretion of pro-Inflammatory Extracellular Vesicles by Oral Keratinocytes

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Objectives Extracellular vesicles (EVs) are involved in several biological processes such as inflammatory response, homeostasis, or wound healing by mediating cell/cell communication. Recently, EVs exhibited potential effect in bone and periodontal destruction. The aim of this study was to isolate and characterize the pro-inflammatory effect of EVs secreted by oral keratinocytes infected by Porphyromonas gingivalis and to assess their inflammatory effects.

Methods Oral keratinocytes (TERT2-OKF-6) were cultured in EV-free medium and infected with P.gingivalis ATCC 33277(MOI:100) for 24h. Differential centrifugation was used to isolate EVs from infected (EVPg) and uninfected (EV) cells. Size and concentration of EVs were determined by nanoparticle tracking analysis (Zetaview). Naïve oral epithelial cells were then exposed to EV, EVPg and P.gingivalis for 24h. EVs’ intracellular internalization was evaluated by fluorescent microscopy. Impact on metabolic activity was evaluated by AlamarBlue assay and protein and gene expression of TNF-a and IL-1β by ELISA assay and RT-qPCR. A miRNA analysis was performed to characterize EVs and EVPg content.

Results EVs isolation from all tested conditions showed that infected cells secreted 10² more EVs than uninfected cells with a diameter comprised between 146 and 161 nm. Fluorescent microscopy confirmed EVs’ endocytosis by epithelial cells. Metabolic assay demonstrated that exposure to EVPg at different concentration significantly increased significantly the metabolic activity of epithelial cells after 24h as observed after P.gingivalis infection. Moreover, ELISA and RT-qPCR analysis demonstrated a significant increase of TNF-a and IL-1β protein and gene expressions in oral epithelial cells exposed to EVPg. We observed an an increased secretion of several miRNAs (HSA-let-7c-5p; HSA-mir-100-5p; HAS-mir-1246) linked to inflammatory pathways activation.

Conclusions This study demonstrated that EVs could play a key role in the inflammatory process and the development of the periodontal lesion associated to P. gingivalis. Further experiments should be performed to evaluate EVs’proteic content and to confirm their role in an in vivo model.

Predictive Role of Periodontitis as Risk Factor for Oral Cancer.

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Objectives Cancer-screening programs need to recognize diversity in the etiopathogenesis of cancer. The present study was done to identify and compare patients with periodontal disease at high risk for developing oral cancer through the expression of molecular proteins associated with cancer in smokers and non-smokers.

Methods Single-centre case-control study enrolled 96 patients; cases: Chronic periodontitis (CP) patients and smokers (n=35) and control: CP patients and non-smokers (n=61). Periodontitis status was recorded through probing pocket-depth (PPD), and clinical attachment level (CAL). Pooled tissue samples were collected from deepest pockets using single curette stroke for detection of anti-apoptotic protein BCL2 through RT-PCR. Samples of unstimulated whole saliva were collected through expectoration to estimate levels of pro-inflammatory oncogenic marker MMP9 using Enzyme-Linked-Immono -Sorbent-Assay (ELISA).

Results Both groups were matched for demographics, PPD, and CAL with no statistically significant difference between cases and control. The results of the present study found expression of BCL2 oncogene and MMP 9 in all the samples of CP patients. However, expression was statistically more in smokers compared to non-smokers. (p<0.5) The real-time PCR results showed that the relative expression of BcL2 was higher in smoking participants than in non-smoking participants with the RQ value 1:2.0508 which shows that the expression of BcL2 within the smoking participant was found to be 2 folds greater than in those of non-smoking participants. MMP9 concentrations were also found to be significantly higher in Smokers (1.997 ± 1.8686) as compared to Non-Smokers (0.999 ± 1.3407) (p=0.001).

Conclusions Smokers do not always develop cancer. CP leads to constant irritation of oral mucosa and may play an important role in oncogenesis. Two times upregulation of BCL2 in cases compared to controls and increased expression of MMP9 in the present study among smokers may be an important predictive marker for future oncogenic activity and requires further exploration.

Point-Biserial correlation of PPD (mm), Average Score (Periodontal pocket depth), CAL (mm), P. gingivalis expression CT values, MMP9 Concentrations and Bcl2 expression CT values with Smoking/Non-smoking
<table>
<thead>
<tr>
<th>Variable</th>
<th>Point-Biserial correlation ($r_{pb}$) with Smoking/Non-smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPD (mm)</td>
<td>-0.175</td>
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<tr>
<td>Average Score (Periodontal pocket depth)</td>
<td>-0.054</td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>-0.184</td>
</tr>
<tr>
<td>P. gingivalis expression CT values</td>
<td>-0.262**</td>
</tr>
<tr>
<td>MMP9 Concentrations</td>
<td>0.299**</td>
</tr>
<tr>
<td>Bcl2 expression CT values</td>
<td>-0.297**</td>
</tr>
</tbody>
</table>

**Correlation is significant at 0.01 level (2-tailed)
The Clinical and Radiological Comparison of NIPSA and MIST: a Retrospective Study
Aylin Oztin, Hasan Gündoğar, Mert Özalpay
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Objectives The main goal periodontal treatment is to stop the progression of periodontal disease and regenerate the lost tissue. Recently, new flap designs have been proposed to minimize surgical trauma. Current techniques have resulted in a reduction in post-operative complications and patient discomfort. The aim of this study is to retrospectively evaluate minimally invasive surgical techniques, MIST and NIPSA in each other.

Methods Thirty-three patients, 12 men and 21 women, age 20-59 years, were included in this study. Areas from 43 surgical procedures in 33 patients were evaluated. The retrospective evaluation was made in 2 groups of patients who have undergone MIST / M-MIST and NIPSA surgery. There are 28 regions where the NIPSA operation is used and 15 regions where the MIST technique is used. In present study, both pre- and postoperative periodontal clinical measurements; plaque index [PI], gingival index [GI], bleeding index on probing [BOP], probing pocket depth [PPD], clinical attachment level [CAL], depth of recession [RC], keratinized tissue height [KT], tip of papillae [TP] were used. The postoperative pain assessment was carried out using the VAS scale.

Results In our study, the PPD and CAL data showed a significant decrease in both groups. There was no statistical difference between the NIPSA and MIST groups for the VAS score. It was observed that postoperative papillary recession was significantly less in the NIPSA group compared to the MIST (p<0.05).

Conclusions Both techniques have shown improved clinical outcomes with minimal patient morbidity. Based on the results of the study, NIPSA is a promising new approach in periodontal reconstructive surgery, particularly in terms of aesthetics, by providing optimal supraalveolar soft tissue stability.

Endo-Periodontal Treatment: Trans-Radicular Release of Active Components by Intracanal Medications
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Objectives In recent studies, endodontic therapy alone, including intracanal medications (ICM), improved periodontal healing in endo-periodontal lesions (EPL) and resulted in probing depth reduction and clinical attachment gain. This study investigated how calcium hydroxide (CH) ICM could induce the release of active components across the dental root, resulting in potential effects on the periodontium. The biological response of periodontal cells exposed to CH- or varying CH+chlorhexidine (CH+CHX) releasing: 0.5%CHX and 1%CHX, were also studied.

Methods Sixty monoradicular teeth were prepared and randomly divided into 3 groups, each of 20 teeth: CH alone; CH+CHX; no ICM (Control). The teeth were immersed in deionized water and the changes in pH as well as the concentration of Ca^{2+} and CHX released into the medium were measured up to 28 days. The effect of different concentrations of CH- or CH+CHX-releasing medium on the viability and mineralization of periodontal ligament fibroblast, cementoblasts, and osteoblasts was evaluated at different time points.

Results ICM resulted in changes in pH and calcium concentration outside the dental root because of diffusion of ions through the apex and the lateral dentinal tubules. Higher amount of ions were released through the apex compared to lateral dentinal tubules (p<0.01). At 7 days, CH+1%CHX had the highest level of hydroxyl (pH increased to 8.18 compared to baseline [6.63]) and calcium ions (concentration increased from 2.42 ppm to 14.67 ppm) compared to CH alone (pH change to 7.74 and [Ca^{2+}] = 10.56 ppm). Overall, exposure of periodontal cells to CH+0.5%CHX releasing media appeared non-toxic, and induced mineralization. CHX release profiles are ongoing.

Conclusions These results might provide insight into the mechanisms behind the positive effect of ICM on periodontal healing in endo-periodontal lesions and suggest that CH based ICM represent potential platform for periodontal drug-delivery.
**O251**

**Temporomandibular Joint Involvement in Patients With Epilepsy**

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**Objectives** This clinical study aimed to assess whether TMD was more prevalent in patients with epilepsy due to increased load to the dentition and the temporomandibular joint (TMJ) caused by seizures.

**Methods** Participants with epilepsy (n=107) and nonepileptic controls (n=100) were enrolled in the study. Those with epilepsy were divided into 3 subgroups (Mild, Moderate, and Severe group) according to their dental manageability. General, dental, and TMJ-related history was recorded, then during the physical examination the range of maximal mouth opening (MMO), possible deviation and deflection, the range of laterotrusion, and the presence of joint noises was registered, following the Diagnostic Criteria for Temporomandibular Disorders. Pressure pain threshold (PPT) was also measured using a digital algometer, on 3 points bilaterally.

**Results** Incidence of TMJ complains did not differ between the control (30%) and the epilepsy group (33%), however, the number of complaints experienced was significantly higher in the epilepsy group (C:3%, E:16% had 3 or more complaints; p <.001). Joint clicking was more prevalent in the entire epilepsy group (p=.012) and in the Mild group (p =.004) compared with controls. Crepitation, joint pain, MMO, laterotrusion, and restricted mouth opening did not show significant difference. Deflection occurred more often (Mild and Moderate groups; p <.001) and in greater extent in the epilepsy subgroups (p <.001) compared with controls. Regarding to PPT, significant difference occurred in Severe group, on the left masseter muscle region’s M1 (p=.046) and M2 (p =.028) points compared with controls.

**Conclusions** As a result of the seizures and the consequent joint overload, the TMJ involvement and the occurrence of TMD symptoms found to be more common or more serious in patients diagnosed with epilepsy. Outcomes of our study support the assumption that epilepsy is truly a risk factor for TMD development.

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**O252**

**Effect of CAD-CAM Abutment Height on Monolithic Zirconia Crown Esthetics**

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**Objectives** The purpose of this in vitro study is to measure and compare color dimensions of different zirconia color shades over two titanium-base abutments heights.

**Methods** One-hundred and sixty identical specimens were milled to restore screw-retained central maxillary incisor crown using (A1/0, A2/3, B2/3 and A3.5/4) color shades 5Y-TZP zirconia blocks. The specimens were milled by using CAD-CAM technology and designed using computer design software to match 3.5 mm and 5.5-mm titanium-base abutments (RC Variobase for crown, Gingival height 1mm, Straumann) (n=20). Color measurements in the CIELab coordinates were made by using a spectrophotometer (VITA EasyShade; VITA Zahnfabrik) under room light conditions. Color difference (ΔE*) values were calculated by using the CIE76 and CIEDE2000 formulas. Perciatibility threshold was set at ΔE00>1.30 and Δab>1.00 units. Clinical acceptability was set at ΔE00>2.25 and Δab>2 units. The data were analyzed by using with 2-way ANOVA and t-test.

**Results** In the A0/1 and A2/3 color groups, ΔE00 and ΔEab showed significant statistical difference between 3.5-mm abutment and 5.5-mm abutment height.

. In group A3.5/4, abutment height increased color components (Δa*, Δb*, ΔL*), and ΔE* variables with no significant statistical differences

**Conclusions** The color results (ΔE>2) of anterior screw retained monolithic zirconia crown over a 5.5-mm titanium-base abutment may result in unacceptable aesthetic outcome when restoring A1/0 and A2/3 color restoration.
O253

Fatigue Analysis of PEEK - Zirconia Hybrid Abutments for Ceramic Implants

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Objectives The aim of this study was to inspect the mechanical fatigue behavior of a ceramic implant system using polyether ether ketone (PEEK) and Zr ceramic as materials for customized hybrid abutments. All-polymer bearings involving polyetheretherketone (PEEK) have been proposed for implant applications. Little is known about the biotribology of polymer PEEK bearings, which are relevant for implant longevity.

Methods Experimental morse taper Zr ceramic implants were included in epoxy resin. PEEK abutments were placed, the specimens were randomly allocated into four groups (injected PEEK or milled PEEK, dry- or saliva stored). To control crack formation in the PEEK/ Zr ceramic zone, we used a methylene blue staining. The specimens were subjected to a fatigue test until procedures were stopped. The load at failure and number of cycles until failure were recorded; survival probabilities and specimen displacement were calculated. The failure pattern was evaluated, and the Weibull modulus was obtained for each condition.

Results A maximum force of 640 N and a minimum force of 64 N was applied to the objects. Fatigue of both types of hybrid abutments was not influenced by the abutment material. In the PEEK abutment, the values obtained in the dry- or saliva stored groups showed no statistical difference. Failure pattern analysis revealed a higher prevalence of cracks for milled PEEK groups, while injected PEEK-group showed a higher crack resistance.

Conclusions The fatigue lifetime is determined through crack initiation processes in the CF regime. It was shown that cracks which cause fatigue failure initiated at phase boundaries between the PEEK- / Zr ceramic surfaces. In the quenched material plastic deformation localizes in slip bands, leading to the formation of growable cracks within those bands inside phase boundaries. PEEK hybrid abutments promoted similar fatigue levels regardless of fabrication methods and of storage mode.
**O254**

**Fit of Thin-Walled Zirconia Veneers Fabricated by 3D-Printing**

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**Objectives** Printing of zirconia is a rather new technology available to fabricate dental restorations. This investigation aimed at identifying the best combination of design and printing parameters with respect to the marginal and internal fit of an upper central incisor veneer.

**Methods** In a pilot study, the optimal printing parameters (exact scaling factors, offset value) for the zirconia material used in 3D-printing (LithaCon 3Y 210) were determined. With this information, the fit (method as described below) of printed veneers was evaluated for different combinations of cement gap design and printing parameters in a second pilot study.

A typodont maxillary central incisor was prepared for a zirconia veneer with 0.5mm wall thickness. This master die was reproduced 24 times by 3D printing (Freeprint Model). Each twelve individual veneers were fabricated by 3D-printing (CeraFab 7500) with the best identified settings (cement gap widths: 20µm at the margin and 60µm for the interior surface) and by milling (Cercon HT, Cercon Brain Expert). A sped-up overnight debinding and sintering process was used for the thin walled veneers. Milled veneers were sintered as recommended by the manufacturer. The veneers were luted (Panavia 21) on the respective dies and sectioned (Isomet HighSpeed Pro) by 3 sagittal cuts and 1 (rather) horizontal cut. Marginal and internal fit were determined from the cross-sections by light microscopy (Smartzoom 5).

**Results** All veneers achieved a mean marginal cement gap width below 100 µm which is often used as a criterion for clinical recommendation. Here, milled veneers (mean/overall maximum marginal gap with: 44±11µm/141µm) performed slightly better than printed veneers (55±9µm/143µm). With regard to the mean internal gap widths, both test groups showed small deviation from the planned value, i.e., 68±14µm for the printed veneers vs. 85±19µm for the milled veneers.

**Conclusions** Zirconia veneers with acceptable fit can be fabricated by 3D-printing.

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**O255**

**Retrospective Follow-Up Study of Monolithic Zirconia Restorations in Bruxer Patients**

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**Objectives** To retrospectively assess the clinical performance of the complete rehabilitation of patients with bruxism treated by teeth- and implant-supported veneered and non-veneered monolithic zirconia restorations with an increased vertical dimension of occlusion.

**Methods** Sixteen bruxer patients, with mean age of 59.5 (± 14.9) years, were treated with 152 veneered and 229 non-veneered monolithic zirconia and were followed up for a mean observation period of 58.8 (± 18.8) months (range: 1–8 years). The patients were clinically and radiographically examined once a year and the clinical data were extracted from the medical records. In the recall appointment, the restorations were evaluated using modified California Dental Association (CDA) criteria. Implant and restoration survival and success rates were recorded and analyzed.

**Results** The cumulative survival rates of implants and restorations were 97.7% and 97.6% respectively. Nine restorations were replaced due to three horizontal tooth fractures, two implant failures and four secondary caries. A total of 43 biologic and technical complications were recorded. In the veneered group, the predominant complication was minor veneer chipping (16.4%), which required only polishing (grade 1) while in the non-veneered group, the predominant complication was open contacts between the implant restorations and the adjacent teeth (14.5%).

**Conclusions** Within the limitations of this retrospective study, we conclude that the survival rates of restorations and implants in patients with bruxism are excellent although veneered zirconia restoration exhibit high rate of minor veneer chipping which required only polishing and the biologic complication of fractured single tooth abutment may occur.
CDA ratings for the non-veneered zirconia group

CDA ratings for the veneered zirconia group
O256

Depth Measuring Accuracy of a Cusp-to-Arch Intraoral Scanning Workflow
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Objectives The aim of this study was to investigate the accuracy of an intraoral scanner using metrology software, in measuring known depths on an artificial tooth, using increasing scanning spans from cusp to full-arch.

Methods A milling machine with a 2mm cutting-diameter bur was used to create increasing depths (T1, T2, T3 and T4) on the mesio-buccal cusp of typodont tooth #17 in an intact maxillary arch of artificial teeth. Different scanning spans of the cusp, tooth, sextant, quadrant, and full-arch were recorded with an IOS (True Definition™, Midmark Corp., USA) taken at baseline (T0) and at T1-T4, and repeated ten times. NCLP was used to validate each increasing cut depth on the cusp. Using superimposition-metrology software, the step-height (μm) of each depth was measured using the cusp, tooth, sextant, quadrant, and full-arch, after aligning and subtracting T0 with T1-T4. The percentage error of IOS’s measurements, compared to NCLP, were analysed using a two-way ANOVA and Tukey’s tests.

Results The depths at T1 to T4, recorded by the NCLP, were 83, 133, 195, and 297μm, respectively. Using the IOS cusp scans, the mean (SD) step-heights at T1-T4 was 85.5(3.3), 130.8(3.2), 192.1(2.4) and 299.4(2.8) μm, respectively; while using the full-arch scans, the step-heights were 35.2(11.1), 109.2(9.8), 173.9(21.7) and 261.1(16.4) μm. As the scanning span increased from cusp to full-arch the percentage error increased (p<0.0001); however, as the depth increased from T1 to T4, this error decreased (p<0.0001). Using the cusp as a reference, the error ranged from -1.7% to +3.5% but increased from -57.4% to -10.9% for the full-arch.

Conclusions The depth measurement errors using an IOS and software-analysis workflow increased as the scanning span increased; however errors remained low using cusp spans and decreased with increasing depth which may be promising for clinical applications quantifying surface change.
Evaluation of an Increased Occlusal Vertical Dimension in a RCT
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Objectives This Randomized Controlled Trial (RCT) aimed to assess the effect an acrylic Removable Appliance (RA) to evaluate an increase of Vertical Dimension of Occlusion (VDO) prior to restorative treatment in patients with tooth wear.

Methods Forty-two patients (36 males, 6 females) with generalized moderate to severe tooth wear and a functional/esthetic problem were included and randomly allocated to 1) no test phase or 2) test phase with RA. Patients were instructed to wear the RA for 24h per day for 3 weeks. Restorative treatment consisted of restoration of all teeth using composite restorations in an increased VDO. Patients were recalled after 1 month and 1 year. Clinical acceptability of restorations, OHIP-score, and freeway-space were assessed at baseline and at recall appointments. Restoration failure was defined as restorations that needed to be replaced, repaired, or refurbished in case of minor materials chippings or when the abutment tooth had increased sensitivity due to occlusal overloading.

Results Clinical follow-up at 1-year recall was completed for 41 patients. In total 1,660 restorations were placed, with 862 restorations placed in the RA-group and 798 restorations placed in the No-RA-group. No significant effect of testing the VDO with a RA could be found on the quality of life after 1 month (p=0.14), or after 1 year (p=0.76). Reduction of freeway-space in the RA group, compared to the control group, was significantly lower at 1 year (p=0.01, 95%CI -1.09 to -0.15).

Conclusions This RCT showed that a removable appliance is not indicated to functionally test the increased VDO prior to restorative treatment in patients with tooth wear.

Early interventions on restorations

<table>
<thead>
<tr>
<th>Restorations in the study (n=1660)</th>
<th>RA (n=862)</th>
<th>No-RA (n=798)</th>
<th>Total</th>
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<tr>
<td>Interventions at recall 1 month</td>
<td>5</td>
<td>3</td>
<td>8</td>
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<tr>
<td>Interventions at recall 1 year</td>
<td>36</td>
<td>23</td>
<td>59</td>
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</table>
Bi-Directional Association Between Oral Health and Cognitive Function
Chenyi Gao¹, Harriet Larvin⁵, Tim Bishop², David Bunce², Sue Pavitt², Jianhua Wu², Jing Kang¹
¹School of Dentistry, University of Leeds, Leeds, United Kingdom, ²University of Leeds, Leeds, United Kingdom

Objectives Oral health (OH) and cognitive function (CF) might be associated as shown by many studies. This cross-sectional study aims to investigate the bidirectional relationship between OH and CF using various statistical approaches.

Methods This cross-sectional study utilized data from the National Health and Nutrition Examination Survey (NHANES 2011-2014). Outcome measures include OH (dentition (missing teeth, MT, 0-28), number of decayed missing and filled teeth (DMFT, 0-28), and periodontal disease (PD, yes/no)) and CF (global cognitive function (GCF, 0-30), memory (0-10), processing speed (PS, 0-10), and executive function (EF, 0-10)). Covariates included demographic, lifestyle, anthropometric, co-mobidities, and dental hygiene behaviour. Bidirectional associations were examined using uni- and multi-variable models on: 1) the effect of OH on CF outcomes (72 uni and multi-variable models); 2) CF’s effect on OH outcomes (72 models); 3) structural equation model (SEM) treating CF and OH as latent variables.

Results 2,504 participants aged 60+ years old were included in the study. 1,987 participants who both completed formal cognitive tests and were dentate were included in analyses. In the fully adjusted models, strong associations between OH and GCF were observed. Especially, better PS predicted better OH outcomes (PD odds ratio OR= 0.88, 95% Confidence Interval (CI) [0.81,0.96], p<0.01; DMFT beta= -0.36, [-0.58, -0.14], p<0.01; MT beta= -0.07, [-0.11, -0.04], p<0.001) and vice versa. In addition, PD is associated with memory, and MT is associated with EF, bidirectionally. The SEM also indicated a strong correlation between OH and CF (r= -0.22, [-0.34, -0.10], p<0.001).

Conclusions This study confirmed the reciprocal association with OH and CF in ageing population. People at older age with poor oral health are more likely to have poorer cognitive function, and people with poor cognitive function are more likely to have poor oral health. The findings suggest future studies should focus on the causal relationship between OH and CF and its biological mechanism.
POSTER PRESENTATIONS abstracts

P100
Life-Threatening Reactions Related to Polyethylene Glycol in Dental Biomaterials
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Objectives The Norwegian Dental Biomaterials Adverse Reaction Unit is responsible for a national reporting procedure concerning suspected clinical biologic adverse reactions associated with dental biomaterials. The procedure is based on spontaneous and voluntary reporting from dentists, physicians and dental hygienists. The reporting is designed to record all types of adverse reactions associated with dental biomaterials. The main objectives of the adverse reaction reporting are to gain knowledge about material-associated adverse reactions and to monitor changes over time. In this study we focused on potentially life-threatening reactions related to polyethylene glycol (CAS No.: 25322-68-3), a chemical which can cause anaphylactic reactions.

Methods Reporting forms are available from the Norwegian Dental Journal, from the internet pages of Norwegian Dental Biomaterials Adverse Reaction Unit, and from the most used electronic patient record software in Norway. Data from received adverse reaction reports were registered in a database. Severe and life-threatening reactions related to materials containing polyethylene glycol were searched for. Information about constituents of the suspected materials was obtained from the instructions for use and safety data sheets.

Results From 1993 to the end of 2021, 2684 reports were received. Of these, three potentially life-threatening reactions were identified. All three were reactions directly after treatment with a temporary endodontic filling material containing polyethylene glycol (PEG 8000). The outcome for one of the patients was fatal, whereas the other two recovered after medical treatment.

Conclusions Life-threatening reactions to materials believed to be safe can be discovered by voluntary reporting to a national reporting system. The mandatory system based on the Medical Device Regulation is this far not a transparent system, and severe reactions could be under-communicated by the manufacturer and notified bodies. Thus, to improve patient safety an open and transparent reporting is warranted and information about the results should be disseminated to dental clinicians.

P102
Correlation Between Phobia of Vomiting, Gag-Reflex, and Dental Anxiety
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Objectives When gagging reflex is activated to minor stimulations it can pose a significant obstacle to routine dental care. Excessive gag-reflex is often attributed as a hidden manifestation of dental anxiety. The aim of the present study was to examine the relationship between phobia of vomiting (Emetophobia), excessive gag-reflex during dental treatment and dental anxiety.

Methods A cross-sectional study was conducted using an online questionnaire, which included the Dental Anxiety Scale (DAS), a questionnaire referring to feelings of nausea and fear of vomiting in various situations (SPOVI- Specific Phobia Of Vomiting Inventory) and questionnaires referring to gag-reflex in different situations related to dental treatment (GAS- Gagging Assessment Scale and GPA-R- Gagging Problem Assessment Revised). The questionnaires were distributed via the web to the general population and to members in a specific forum dealing with emetophobia.

Results 164 subjects completed the questionnaires (19 men, 144 women, and one defining his/her gender as "other"; mean age 34 ±11). 20% of the respondents (N= 32) suffered from excessive gag-reflex (GAS>9; GPA-R>17) and reported feelings of nausea and a desire to vomit in various experiences related to dental treatment. 39% of the respondents (N=64) reported severe dental anxiety (DAS>13) and 62% (N=102) suffered from emetophobia (SPOVI >9).

SPOVI scores correlated significantly with both DAS and GAS (r=0.287 and 0.51, respectively, p<0.001 each); while GAS and DAS scores correlated with each other (p<0.001; r=0.51) (Pearson correlation coefficients). The odds of a subject with an excessive gag-reflex to be diagnosed with emetophobia increased by 11.5 as compared to an individual with no excessive gagging (Chi square).

Conclusions An excessive gag-reflex is closely associated with both dental anxiety and emetophobia.
**P103**

**Persistent COVID-19 Oral Manifestations and Oral-Health Quality of Life**

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**Objectives**

To determine the prevalence of persistent COVID-19 oral manifestations in patients that were hospitalized during the first pandemic wave in Spain and their impact on the oral health-related quality of life.

**Methods**

This cross-sectional study included 113 patients who were discharged after hospital admission due to acute COVID-19 at the time of the first pandemic outbreak in Spain (March-April, 2020). The protocol of the study was approved by the Research Ethics Committee of the Rey Juan Carlos University and the participants signed a specific informed consent. All patients were interviewed by a trained dentist and data collection was carried out through a questionnaire divided in the following blocks: taste alterations, olfactory dysfunction, dry mouth, salivary gland disorders, temporomandibular joint disorders, facial pain, and others (halitosis, ulcers,...). The Oral Health Impact Profile-14 (OHIP-14) questionnaire was used to evaluate the oral health-related quality of life. The data was descriptively analyzed (IBM, SPSS).

**Results**

Of the total number of patients examined 42% had some type of oral disorder related to persistent COVID-19. The most common complaints after 18-month hospital discharge were gustatory disorders (8.8%), olfactory disorders (15.9%), xerostomia (21.2%), and temporomandibular joint disorders (7.1%). During the acute phase of the disease 50.4% and 44.2% of the patients reported gustatory and olfactory alterations, respectively. The prevalence of the remaining oral manifestations collected (facial pain, ulcers, halitosis, etc) were less than 5%.

The presence of oral manifestations attributed to COVID-19 were not related with an alteration in the quality of life in any of the dimensions (functional limitation, physical pain, psychological discomfort, physical disability, psychological disability, social disability and handicap).

**Conclusions**

In conclusion, olfactory and gustatory disorders were the were the most frequent manifestations in patients with persistent symptoms of COVID-19 with no repercussion on their quality of life.

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**P104**

**Immigrant Parents’ Knowledge and Attitudes Related to Children’s Sugar Snacking**

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**Objectives**

Aim: The socio-demographic distribution of parental attitudes and knowledge related to children’s oral care is less known among immigrants in Norway. Focusing immigrant parents with children aged 0-6 months, this study evaluated whether awareness about and attitudes towards restricting children’s sugar intake vary according to family socio-demographic background and parents’ oral health behaviors.

**Methods**

A cross-sectional study was conducted including immigrant parents of western and non-western background attending child public health centers for vaccination of their children. Cross-tabulation and multiple variable logistic regression were used to analyze the data.

**Results**

A total of 345 parents (response rate 72.6%) completed personal structured interviews at the health centers. Mean age of mothers and fathers were 30.4 years and 35.5 years. Overall attitudes and knowledge related to children’s sugar restriction were socio-demographically unequally distributed among immigrant parents. Parents from Asia/Africa presented with more frequent sugar intake than their East European and North American-European counterparts. Employed mothers, mothers with immigrant background from North America/Europe and parents with less frequent own sugar intake were more likely to confirm positive attitudes towards control of children’s sugar snacking. Corresponding odds ratios were OR=1.8 (95% CI 1.1-3.1), and OR=6.6 (95% CI 2.3-18.9). Mothers with longer than shorter stay in Norway, having immigrant background from East Europe compared to Africa/Asia and those who confirmed receipt of dental care information were more likely than their counterparts to present with good oral health knowledge.

**Conclusions**

Socio-demographically less affluent parents were less likely to report positive attitudes and to have good knowledge regarding control of children’s sugar snacking. Information about modifiable oral health indicators might be used to inform interventions targeting immigrant parents most at risk. Culturally adapted oral health intervention programs should be implemented for immigrants, with special reference to children’s dietary habits.
**P105**

**Development of a gum-Related Quality of Life Measure**

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**Objectives** Gum health studies have often not considered impacts from across the entire gum health-disease continuum, from gingivitis through to periodontal disease. Use of existing oral health-related quality of life measures also limits measurement of more specific gum health-related impacts that individuals experience. The aim of this study was to develop a new gum-related quality of life measure using qualitative data on the everyday impacts of a range of gum symptoms.

**Methods** Twenty-seven participants were recruited (15 female, 12 male; 23-73 years) with a range of symptoms associated with gingivitis (n=15) and periodontal disease (n=12). Semi-structured interviews explored experiences with symptoms and associated impacts. Framework analysis was used to map findings onto the Wilson and Cleary (1995) health-related quality of life model, from which representative domains and items for the new measure were selected. Following this, a preliminary pilot validation study tested the measures wording and relatability.

**Results** Greater impacts were reported by those with periodontal disease (severity, extent and frequency of symptoms) than with gingivitis. However, participants from across the continuum expressed similar concerns, with participants with gingivitis also reporting impacts on daily life. The domains (and associated number of items) of the new 64-item gum health experience questionnaire (GHEQ) were: symptoms (n=17), changes in everyday life (n=13), social impacts (n=5), psychological/emotional impacts (n=11), identity (n=5), and overall impacts and quality of life (n=7). A section on the timeline of treatment and symptoms was also added (n=6).

**Conclusions** This research demonstrates the range of impacts associated with gum health. The development of a person-centred measure aiming to capture everyday experiences of individuals with a range of symptoms could be helpful in aiding with communication in clinical settings and treatment plans. Further validation and testing of the measure is required before it can be used in research or clinical settings.

**P106**

**Access to Orthodontic Treatment Among Children by Household-Income in Norway**

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**Objectives** To examine income-related inequalities in access to orthodontic treatment among children in Norway. The cost of orthodontic treatment is high. Therefore, the National Insurance Administration refunds part of the cost of treatment for children under 18 years of age. A policy goal is that access to orthodontic treatment shall be equal to everybody regardless of household income.

**Methods** The analyses were carried out using data from the Norwegian Health Economics Administration and Statistics Norway. All persons who live in Norway have a unique personal identification number. This made it possible to merge the data from the Norwegian Health Economics Administration with data from Statistics Norway. Our data file encompasses the whole population of individuals 6 to 18 years from 2013 to 2019 (n = 790 355). The analyses were carried out using logistic regression and ordinary least square regression. The dependent variables were whether an individual received orthodontic treatment or not, and how much reimbursements each individual received. The key independent variable was household income. The control variables were: age, gender, parents’ highest education, place of birth, county of residence.

**Results** Household income had a positive effect on the probability of receiving orthodontic treatment and reimbursements. The lowest income group was the reference group. The coefficients of receiving orthodontic treatment according to income increased from 0.022 (95% CI = 0.018-0.026) in the second-lowest income group to 0.07 (95% CI = 0.063-0.072) in highest income group without adjustments. The coefficients of reimbursements also increased from 0.11 (95% CI = 0.07-0.16) in second-lowest income group to 0.31 (95% CI = 0.27-0.36) in the highest income group with adjustments.

**Conclusions** There are inequalities in access to orthodontic services according to household income. Children from the low-income group are less likely to have orthodontic treatment and they have lower reimbursements.
Interest in Oral Cancer on Internet in Europe

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Objectives Tobacco and alcohol are the main risk factors for oral cancers (OC), the low survival rate of which is a public health problem. European-wide health policies (a prevention campaign, tobacco packaging) have been put in place to inform the population of the risks associated with consumption. The identification of internet research data on the population could help to measure the impact of and better position these preventive measures. The objective was to analyze a potential temporal association between public health programs and interest in OC on the internet in the European Union.

Methods A search of data from Google ©, Wikipedia © and Twitter © users in 28 European countries relating to OC between 2004 and 2019 was completed. Bibliometric analysis of press and scientific articles over the same period was also performed. The association between these data and the introduction of public health programs in Europe was studied.

Results There was a temporal association between changes in tobacco packaging and a significant increase in internet searches for OC in seven countries. Unlike national policies and ad campaigns, the European awareness program Make Sense has had no influence on internet research. There was an asymmetric correlation in internet searches between publications on oral cancer from scientific articles or “traditional” media (weak association) and those from internet media such as Twitter © or Wikipedia © (strong association).

Conclusions Our work highlights seven areas around which OC awareness in Europe could be refocused, such as a change in the communication of health warnings on cigarette packs, the establishment of a more explicit campaign name regarding OC, the involvement of public figures and associations in initiatives to be organized at the local level and the strengthening of awareness of the dangers of tobacco in the development of OC.
Biocompatibility, Physical and Chemical Analysis of Calcium Silicate-Based Endodontic Sealers
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Objectives The present study aimed to evaluate, in vivo, the biocompatibility and in vitro, physical, and chemical properties of a newly proposed pre-mixed material in comparison with two other calcium silicate-based sealers and an epoxy resin-based material.

Methods Bio-C Sealer Ion+, Bio-C Sealer, EndoSequence BC Sealer, and AH Plus (as control) were evaluated for subcutaneous inflammatory response after animal implantation. After 7 and 30 days, the rats were euthanised and the tubes were removed along with the surrounding tissues for the histologic analysis. Material surface and chemical characterisation were analysed using scanning electron microscopy (SEM), energy dispersive spectrometry (EDS) and Raman spectroscopy. According to ISO 6876/2012 standard, flow, setting time (in both dry and moist environments), solubility, radiopacity, and pH were also analysed. The parametric results were statistically analysed by ANOVA using Levene test and Bonferroni correction and the non-parametric by Kruskal-Wallis followed by the Dunn test (p<.05).

Results The inflammatory response observed for the calcium silicate-sealers was greater after 7 days subsided after 30 days. Peaks of calcium, silicon, and radiopacifier were observed for the calcium silicate-based sealers in the SEM/EDS and Raman analysis. All materials exhibited flow values above 17 mm, fulfilling the ISO standard. Bio-C Sealer obtained the shortest setting time in both environments; however, regarding the solubility, Bio-C Sealer Ion+ exhibited similar results of those obtained for EndoSequence BC Sealer and Bio-C Sealer (>0.05) not fulfilling the ISO standard. All materials exceeded 7 mm Al of radiopacity and exhibited a decreasing alkalinity until 21 days.

Conclusions Bio-C Sealer Ion+ exhibited comparable properties to the other pre-mixed sealers, including their high solubility not fulfilling the ISO standards. Long-term endodontic sealers stability should be expected to ensure successful clinical outcomes, thus high solubility is a major concern considering pre-mixed ready-to-use sealers.

Fracture Resistance of CAD/CAM Ceramic Restorations With Different Cementation Strategies
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Objectives The type and thickness of CAD/CAM ceramic restoration might influence fracture resistance along with the cementation strategies. This study aimed to evaluate the influence of ceramic types, thickness, and cementation strategy on fracture resistance of CAD/CAM ceramic cemented on dentin.

Methods Ninety-six extracted non-curious human third molars were collected and flattened to dentin surface from occlusal. The specimens were randomly divided into twelve groups (n=8) according to CAD/CAM ceramic restorations type(Celtra Duo and IPS e.max CAD), with 0.8-mm and 1.5-mm disk-shaped thickness, and three different cementation strategies: 1) SBU/ULT(AL): Auto-curing on the adhesive (Single Bond Universal; SBU) and light-curing on cement (RelyX Ultimate; ULT), 2) SBU/ULT(LL): Separately light-cured on adhesive and cement, and 3) Composite cement: Light-curing on two-step self-etch adhesive (Clearfil SE Bond) and light-curing on composite (G-aenial Anterior). The cement was applied onto the ceramic disk and placed on the dentin surface, then kept under humidity conditions before putting into the universal testing machine. The fracture load was indented perpendicular to the ceramic surface until the ceramic was fractured. The fracture loading (N) was statistically analyzed using three-way ANOVA and Tukey's Post-Hoc test (a=0.05).

Results The type of ceramic material, ceramic thickness, and cementation strategy were statistically significant at p<0.05. The 1.5-mm thickness IPS e.max CAD with SBU/ULT(LL) and composite cementation performed the highest fracture resistance, while the 0.8-mm thickness Celtra Duo with SBU/ULT(AL) were the lowest. The cementation with SBU/ULT(LL) and composite cement performed better fracture resistance than SBU/ULT(AL).

Conclusions A separately light-curing on the adhesive and the light-curing on the dual-cure resin cement (‘LL’ mode) has been recommended for adhesive cementation to enhance the fracture resistance of ceramic, which was comparable to ceramic cemented with light-cured adhesive and restorative composites.
Micro-Tensile Bond Strength of Zirconia and Lithium disilicate Manufactured by Printing and Milling

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Objectives The aim of the in-vitro study was to evaluate the bond strength between dentin and additive or subtractive manufactured Zirconium dioxide(ZrO) and Lithium disilicate(LiSi) by micro-tensile test (µTBS).

Methods ZrO and LiSi were LCM printed and milled in the Dimension of 2x2x8mm³. On human 3rd Molar’s enamel was removed and the dentine was sliced in rods of 2x2x8mm³. The ZrO was sandblasted by Al₂O₃ (50µm, 1bar) and the LiSi were etched by hydrofluoric acid for 20s, multifunctional Silan with MDP was applied (Monobond Plus). Dentin was prepared with Dentin Bonding adhesive (Syntac Classic) and specimens were fixed with composite (Variolink Esthetic DC) and light cured for 40s. Specimens were aged by 10000 thermocycles. The specimens were fixed to the testing machine (tc-550m) and stressed in tension at a crosshead speed of 0.5 mm/min. µTBS values in MPa were obtained by dividing the force at failure by the cross-sectional area of each specimen. Failure mode was observed using light microscopy and representative pictures were made by FE-SEM. Shapiro wilk test and unpaired t-test has been applied for statistical analysis of differences between experimental groups using a level of significance of p<0.05 and Weibull’s analysis was done.

Results µTBS varied between 5.88±2.22MPa and 6.34±2.26MPa. No significant difference could be observed between the manufacturing method or material groups. Printed ZrO showed the highest reliability with m=2.85. The overall failure pattern was cohesive in composite.

Conclusions The choice of manufacturing method and material is negligible in view of µTBS. The new printed materials preformed as good as the conventionally milled once.

<table>
<thead>
<tr>
<th>µTBS [MPa]</th>
<th>printed</th>
<th>milled</th>
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<tr>
<td>Zirconia</td>
<td>5.88±2.22</td>
<td>6.30±2.74</td>
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<tr>
<td>LiSi</td>
<td>6.34±2.26</td>
<td>6.26±2.42</td>
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Effect of Firing Process on Accuracy of Machinable LDS Blocks
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Objectives A novel lithium disilicate (LDS) glass ceramic, Initial LiSi Block does not require the crystallization process after CAD/CAM fabrication. On the other, staining and glazing are applicable as one of options if required. The objective of the study is to evaluate the accuracy of the geometric crown before/after firing process.

Methods Three LDS glass ceramic blocks shown in the table were tested. The geometric crowns were fabricated from these glass ceramic blocks using CEREC MC XL (Production repeatability: ±25µm, Dentsply Sirona). The obtained crowns ware crystallized or glazed according to manufacturers’ instructions. Then, the accuracy was compared by superimposing the crowns and the original STL data. The accuracy was defined as percentage within the production repeatability (±25µm). Therefore, higher values indicate better accuracy. The data was analyzed with one-way ANOVA and Tukey’s tests (p< 0.05).

Results The table shows the results. Same superscripts indicate no significant difference. LS was the most accurate both before/after glazing process.

Conclusions EM requires crystallization at the temperature above the deformation point as shown in the Table. Therefore, we considered that the dimensions were deformed during crystallization. CT was no significant difference before/after glazing process. However, we found CT has the lower accuracy values and the large standard deviation. CT is took longer grinding process time. This means CT is difficult to grind and it may affect to accuracy. Thus, CT is considered that it could not be grinded it accurately in accordance with the STL data.

The accurate workpiece was fabricated from LS because LS has good grinding properties by CAD/CAM machines. In addition, LS is stable under heat treatment for glazing.

Table.

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<tbody>
<tr>
<td>Initial LiSi Block (LS, GC Corp.)</td>
<td>750 / 792.8±4.7</td>
<td>Before Firing</td>
<td>63.2±3.9a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After Firing</td>
<td>63.7±0.9a</td>
</tr>
<tr>
<td>IPS e.max CAD (EM, Ivoclar Vivadnet)</td>
<td>840 / 809.8±2.4</td>
<td>Before Firing</td>
<td>53.6±3.3a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After Firing</td>
<td>27.2±1.4b</td>
</tr>
<tr>
<td>CEREC TESSERA (CT, Dentsply Sirona)</td>
<td>760 / 788.4±2.6</td>
<td>Before Firing</td>
<td>25.0±10.3b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After Firing</td>
<td>25.0±7.6b</td>
</tr>
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P113
Effects of Different UV-C Irradiations on Dental Zirconia
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Objectives The aim of this study was to study the surface effects of two ultraviolet(UV)-C irradiations, 222nm and 254nm, on dental zirconia.
Methods A total of 12 blanks of zirconia milling blanks (3M ESPE Lava Frame; 3M ESPE AG., Germany) were used and cut into squared pieces using a low-speed diamond saw (Buehler Isomet, USA) that yielded a final dimension of 10.0mm x 10.0mm x 1.2mm after sintering (ZYCOMAT 6000 MS, Germany) for about five hours. Seventy-two specimens were ultrasonic cleansed, and divided into 24 groups such that half of them were irradiated with 222nm UV-C (Excis2-250-80W) and another half were irradiated with 254nm UV-C (ZW8S 15Y-Z288) fluorescence light sources, varying with different distances (1cm, 6cm and 12cm) and irradiation time (40mins, 120mins, 480mins and 1440mins). Three additional untreated specimens were retained as control. Then, a colour spectrophotometer (NR10QC, 3nh) was used to measure the colour change values (ΔE) of zirconia surfaces at three different positions after ultraviolet irradiations. Water contact angles (WCA) were measured with goniometric approach using ImageJ. SEM and EDX were used to capture the surface morphologies and Zr/O ratios, respectively. Statistical analyses were carried out by Statistical Package for Social Science (SPSS, Version 26, IBA, Armonk, New York, USA) at 5% significance level.
Results Irradiating 222 nm or 254 nm UV-C on zirconia able to change the colour of zirconia with respect to time according to power-law relationship, such that 6cm and 1cm for 222nm and 254nm irradiation, respectively, change colour the most. For WCA, 3-way ANOVA revealed wavelengths and irradiation time are significant (p<0.001) influencing factors, such that the WCA were found to be linearly correlated with log(irradiation time) and 222nm yielded significantly lower WCA than 254nm. No microscopic changes were observed in SEM, but both UV-C irradiations would significantly increase (p<0.05) the Zr/O compare with control group.
Conclusions Both 222 nm and 254 nm UV-C were able to change the colour of dental zirconia and increase WCA under different time due to the oxygen deficiency mechanism.

P193
Low-Temperature Degradation of Surface-Treated 3Y, 4Y and 5Y Zirconia Ceramics
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Objectives Air-particle abrasion (APA), used to promote adhesion to resin cement, adversely affects zirconia’s surface integrity which is more critical in newer translucent zirconia ceramics. Likewise, surface low-temperature degradation (LTD) of zirconia in a humid oral environment may compromise the materials’ strength and reliability. As in mechanical and optical properties, differences can be found in the susceptibility of different yttria-containing zirconia ceramics (YZ) to LTD. This study aimed to evaluate the effects of APA and LTD on mechanical and crystallographic properties of different YZ.
Methods Four zirconia powders stabilized with 3-5 mol.% of yttria were dry-pressed to form disc-shaped specimens (n=90/gp). After sintering (1450°C, 2 h), control specimens were left in as-sintered (AS) state (n=30/gp) and test specimens were surface-treated with APA (50 µm Al2O3, 2 bars; n=60/gp), of which 30/gp were submitted to regeneration firing (RF; 1000°C, 15 min). Subsequently, all specimens followed accelerated LTD in an autoclave (134°C, 2 bars, 48 h). Biaxial flexural strength was determined (ball-on-three-balls method; 1 mm/min) coupled with Weibull analysis. XRD and FIB-SEM analysis determined crystal phases and transformation zone depth (TZD), respectively. ANOVA with Tukey’s post hoc test was used (p<0.05).
Results APA increased the amount of monoclinic phase in the 4 and 5 mol.% yttria zirconia (4Y, 5Y) after accelerated LTD compared to AS counterparts but did not affect the characteristic strength (σ0) and reliability (m), as was confirmed by the XRD results. For 3 mol.% groups (3Y), RF significantly lowered the amount of monoclinic phase after accelerated LTD and increased the σ0 and m. FIB-SEM revealed various patterns of TZD.
Conclusions APA adversely affected the LTD process of 4Y and 5Y zirconia, however, it did not deteriorate its strength and reliability. Notably, RF after APA of 3Y appeared to slow down the LTD process and increased the materials' strength and reliability.
Fracture Patterns of NiTi Endodontic Files After Clinical Use
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Objectives Fracture of NiTi rotary instruments has a direct impact on the negative outcome of an endodontic treatment. Therefore, the aim of this study is to examine and compare the fracture plane of discarded MTwo and Rotate NiTi rotary instruments following use in root canal treatment.

Methods A total of 221 instruments including 105 MTwo (conventional NiTi alloy) and 116 Rotate (thermally treated NiTi alloy) that failed due to either fracture or deformation was collected for examination. The failed parts of the instruments were examined under an operation microscope (Zeiss, Germany) and categorised by their designated failure behavior, such as plastically deformed but not fractured, fractured with plastic deformation, and fractured without deformation. Additionally, SEM images taken under x1.00 K, x2.5 K and x5.00 K magnifications were evaluated to characterise each instrument’s fracture pattern.

Results Macroscopic evaluation with the operation microscope showed that within all the discarded instruments, 9% were deformed without fracture, 11% were plastically deformed with fracture, and 80% were fractured without plastic deformation. MTwo files exhibited a larger number of fractures with no evident deformation (83.8%), whereas Rotate instruments included more files presenting permanent deformation (76.7%). SEM images revealed failure patterns of examined instruments that were of a ductile character with shallow dimples elongated through the fracture plane for fracture without plastic deformation whereas no surface dimples were seen for instruments fractured with plastic deformation.

Conclusions SEM analysis showed that single overloading causing a ductile fracture is more common in systems manufactured of conventional Ni-Ti alloy compared to thermally treated ones. Clinicians should be aware of these possible behaviors to avoid unwanted fractures.
P115
Characterization of the new Titanium Alloy Ti-20Zr-3Mo-3Sn for Dental Implants
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1Univ Rennes, INSA Rennes, CNRS, ISCR UMR 6226, Rennes, France, 2CHU Rennes, Center of odontology and oral surgery, Rennes, France

Objectives In the field of metallic biomaterials, and more particularly for titanium-based alloys, improvements in biocompatibility have been made in recent years to optimize the properties of medical devices. In oral implantology, particular attention has been focused on some β titanium alloys thanks to their modulus of elasticity which is generally lower than α and (α + β) type alloys. In this study, the new metastable β titanium alloy Ti2033 (Zr: 20% at, Mo 3% at and Sn: 3% at), was compared to the reference alloys Ti-6Al-4V and Ti-14Zr currently marketed for dental implants.

Methods The Ti2033 alloy was synthesized by cold crucible levitation melting technique, was cold rolled and was punched into discs for biological evaluation. Then, specimens were finally treated in order to retain the β-phase in its metastable state. Tensile tests were performed to evaluate the mechanical properties of the samples. Cell viability assays were done using an MTT test with human-derived osteoblasts (SaOs-2) at 1, 3 and 7 days.

Results The main mechanical characteristic of this alloy is a modulus of elasticity almost twice lower than conventional titanium alloys used in oral implantology and one of the lowest modulus of elasticity among β titanium alloys developed for medical applications. From a biological point of view, in vitro cell viability (SaOs-2) is similar (no significant difference) to the reference alloys Ti-6Al-4V and Ti-14Zr.

Conclusions Given the mechanical properties of the Ti2033 alloy and the encouraging results of studies carried out on Ti-Zr alloys, this new alloy seems to be suitable for biomedical use. Other studies are still necessary to validate its first results, and functionalization tests are in progress.

P116
The Ex-Vivo Study of Bioactive Glasses in Different Carriers
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1Department of Operative Dentistry and Endodontics, Mahidol University, Faculty of Dentistry, Bangkok, Thailand, 2Dental Biomaterials Science, Mahidol University, Faculty of Dentistry, Bangkok, Thailand, 3Department of Oral Biology, Mahidol University, Faculty of Dentistry, Bangkok, Thailand, 4Department of Pharmacology, Mahidol University, Faculty of Dentistry, Bangkok, Thailand, 5Department of Oral and Maxillofacial Pathology, Mahidol University, Faculty of Dentistry, Bangkok, Thailand, 6Assistive Technology and Medical Devices Research Center, National Science and Technology Development Agency, Bangkok, Thailand

Objectives The study evaluated the pulpal response of different bioactive glasses and TCP incorporating UDMA or Fibrinogen-Thrombin in the human tooth culture model.

Methods Bioactive glasses (45S5F, SS3P4) and TCP were mixed with different carriers (UDMA or Fibrinogen-Thrombin), and Biodentine was used as a control. The experimental pulp capping materials were evaluated using the human tooth culture model. The histology and immunohistochemistry were assessed at 2 and 4 weeks. The mineralisation foci data were analysed using Kruskal-Wallis H tests and Mann-Whitney U tests.

Results Mineralisation foci were significantly higher at four weeks for Biodentine and the experimental materials (45S5F, SS3P4, TCP) in Fibrinogen-Thrombin than UDMA (p=0.001). Biodentine had the highest mineralisation foci among the other materials at four weeks.

Conclusions Fibrinogen-Thrombin showed a better medical carrier for pulp capping material to UDMA. These bioactive glasses were a promising candidate for pulp capping materials.
P117
Promising Pro-Osteogenic Effect of a Novel Nanoemulsion Loaded With Hesperitin
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1Physiology and Pathology, School of Dentistry, São Paulo State University (UNESP), Araraquara, São Paulo, Brazil, 2School of Dentistry, São Paulo State University (Unesp), Araraquara, Brazil, 3School of Pharmaceutical Sciences, São Paulo State University, Araraquara, SP, Brazil

Objectives The aim of the present study was 1) to develop a nanoemulsion loaded with hesperitin (NE-HT), and 2) evaluate in vitro, the effect of this novel nanoemulsion on proliferation, differentiation and metabolic activity of osteoblasts.

Methods The nanoemulsion loaded with hesperitin was synthesized and characterized by dynamic light scattering (DLS) through Zetasizer equipment. To evaluate the bioactivity of the novel nanoemulsion, human osteoblast like SaOs-2 cells were treated with different concentrations of NE-HT to determine its cytotoxic effect by MTT (Methyl-Thiazolyl-Tetrazolium) assay. Cell proliferation was evaluated by Alamar blue assay. Additionally, osteogenic differentiation was evaluated by the alkaline phosphatase (ALP) assay and detection of calcium deposits with alizarin red staining (ARS).

Results A novel NE-HT exhibited the droplet size less than 98.90 ± 1.30 nm, polydispersity index less than 0.58 ± 0.03 and zeta potential greater than -38.03 ± 0.80 mV after 1, 7, 14, 21 and 90 days of evaluation. Non-cytotoxic concentrations of NE-HT enhanced cell proliferation and promote osteogenic differentiation by ALP activity and mineralization nodule formation. NE-HT showed a dose-dependent effect.

Conclusions Overall, this in vitro study suggests that nanoemulsion loaded with hesperitin has a pro-osteogenic potential in osteoblasts culture and it can be considered a promising drug for bone regeneration of periodontal disease.

P119
Cytotoxicity, Debris and Smear Layer Removal Efficacy of Super-Oxidized Water
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1Institute of Dentistry, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, 2Life Sciences Center, Institute of Biochemistry, Vilnius University, Vilnius, Lithuania

Objectives The present study evaluated the cytotoxicity of super-oxidized water (SOW) on cultured human gingival fibroblasts and its efficacy in debris and smear layer removal from root canal walls.

Methods Human gingival fibroblasts were grown from the connective tissue graft and exposed to SOW (Sterilox), which was diluted in the medium at 30%, 40%, 50%, 60%, 70% concentrations. The same range of dilutions of sterile distilled water served as a control. Cell viability was evaluated by MTT assay after incubation period of 1h, 2h, 24h and 48h. Pathological cellular changes were also observed under fluorescence and phase contrast microscopes. Efficacy in debris and smear layer removal was evaluated in comparison to the conventional application of sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA). Forty maxillary premolars having single root canal were randomly divided into two groups (n=20) and shaped with ProTaper NEXT instruments using Sterilox or NaOCl/EDTA for irrigation. Roots were split longitudinally and examined under scanning electron microscope at ×200 and ×1000 magnifications. Statistical analysis was performed with Shapiro-Wilk, Levene’s and ANOVA tests (p<0.05). Smear layer and debris scores having non-normal distribution were compared using Mann-Whitney (inter-group comparison) and Wilcoxon (intra-group comparison) tests.

Results Cell viability diminished in concentration and time dependent manner, reaching the lowest percentages of viable fibroblasts after 48h at 60% and 70% concentrations. However, significant decrease in cell viability was observed only at highly concentrated SOW, whereas concentrations up to 50% retained acceptable biocompatibility within the specified time periods. No significant differences were detected between SOW and NaOCl/EDTA in debris and smear layer removal.

Conclusions SOW exhibits acceptable biological properties for endodontic application at concentrations up to 50%, and is equally effective to the conventional irrigation protocol of NaOCl/EDTA in terms of debris and smear layer removal.
Hesperitin-Loaded Nanofibers Suppresses Inflammatory Response of Macrophages and Keratinocytes

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Objectives: In this study, hesperitin-loaded nanofibers were developed and their effects on inflammatory response in a macrophage and keratinocytes models stimulated with Fusobacterium nucleatum were evaluated. Moreover, activation of the NF-κB and Phospho-p44/42 signaling pathways were investigated.

Methods: Nanofibers of PLLA (poly-L-lactic-acid) loaded with hesperitin were developed by the electrospinning technique for drug delivery. The fiber diameters and eventual artifacts were evaluated by scanning electron microscopy (SEM). The inflammatory response of this hesperitin-loaded nanofibers was evaluated using macrophages and keratinocytes seeded on nanofibers and stimulated with F. nucleatum. Levels of pro-inflammatory cytokines and matrix metalloproteinases (MMPs) were determined by enzyme-linked immunosorbent assay (ELISA). Additionally, activation of the NF-κB and Phospho-p44/42 signaling pathways were monitored using a luminescence assay and ELISA, respectively.

Results: Hesperitin-loaded nanofibers appeared randomly oriented and thick, and showed small interfibrillar spaces. In vitro experiments showed that hesperitin-loaded nanofibers were able to significantly inhibit the production of IL-6, IL-8, IL-1β, TNF-α, MMP-2, and MMP-9 by macrophages stimulated with F. nucleatum. Hesperitin-loaded nanofibers also significantly decreased IL-6 and IL-8 levels produced by keratinocytes. Lastly, hesperitin significantly attenuated F. nucleatum-mediated activation of NF-κB and Phospho-p44/42 signaling pathways.

Conclusions: Although clinical trials are necessary, the results of the present study suggested that nanofibers loaded with hesperitin may be a potential therapeutic biomaterial for the treatment of periodontal disease if used in a local drug delivery system due to its anti-inflammatory effect through inhibition of NF-κB and Phospho-p44/42 signaling.
**Strategies to Optimize Bone Regeneration in 3D Printed Scaffolds**

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**Objectives**

3D printing technologies facilitate the design of personalized calcium-phosphate based bone substitutes. Our team has developed a self-setting calcium phosphate ink combining reactive ceramic particles with hydrogel binder, that hardens through a dissolution and precipitation process at low temperature. The control of the reaction’s duration adjusts the crystal’s morphology during the hydrolysis process, thus altering its physicochemical properties. The final product is a nanostructured apatite very similar to the mineral phase of bone. This self-setting ink could then be printed in implants constituted by strands of different profiles (cylindrical or star-shaped). The ink’s curing kinetic could be modified by biomimetic or hydrothermal methods. Our objective was to analyze the influence of the nanostructure, nanoporosity and nanopore size of the two curated apatite inks, as well as of the shape of the printing strands, in its osteogenic potential in vivo.

**Methods**

Two studies were conducted in rabbit condyle monocortical defects: one comparing 3D printed a-TCP scaffolds hardened by biomimetic (Bs) versus hydrothermal (Hs) reaction, and the second comparing hydrothermal-hardened scaffolds constituted by cylindrical strands versus star-shaped strands.

**Results**

The Hs showed a higher nanoporosity (57.1%) than the Bs ones (48.4%) as well as a larger pore entrance size (0.062 μm versus 0.017 μm). The BSA adsorption pattern of the Hs was also significantly higher than the one from the Bs. The morphological features revealed by BS-SEM and Histology revealed than the osseous tissue in the Hs was more mature than in the Bs. When comparing the SEM and histological observations of the different ink strands, the star-shaped ones showed a higher level of osteoconduction into the scaffold structure than the cylindrical ones.

**Conclusions**

Hydrothermal treatment improves the ink nanotexture and osteoregenerative effect while the concavity of the filament’s surface, increases bone osteoconduction, proving to be a good strategy to optimize 3D printed personalized bone grafts.
**Characteristics and Biological Considerations of Newly Developed NanoFA-Based Dental Composite.**

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**Objectives**

The objectives of this project were to develop fluoride releasing model dental composites incorporating nano-fluorapatite (nanoFA) as secondary fillers and to measure their degree of conversion (DC) as well as fluoride ion release. Moreover, the toxicity of the monomers UDMA, HEMA was investigated. The overall effect of the new composite material on gingival fibroblasts was assessed by RNA-Seq analysis.

**Methods**

Composites containing UDMA: HEMA (4:1) as resin (37.9% vol) with silica glass (62.1% vol) and nanoFA as filler part (20% weight) were made. G-ænial Anterior (GC Europe) was used as comparator. The DC was recorded by FTIR, and an ion-selective electrode was used to measure fluoride release for a period of one month storage. Monomer toxicity was tested by colorimetric XTT viability assay. The underlying cellular mechanisms were characterised by gene expression analysis using RT-qPCR and RNA-Seq analysis with gingival fibroblasts exposed for 24 hours to the dental composites.

**Results**

The DC for the new material was 84 % and after 28 days 2.5 µg/(cm$^2$.day) fluoride was released compared to the comparator. The toxicity studies revealed a significant reduction in the cell viability at 2 mM of HEMA and 1 mM of UDMA. RT-qPCR showed a dose dependent expression increase of DNA repair genes (DDX11, IPPK, XRCC2 and RAD50) in gingival fibroblasts treated with HEMA. RNA-Seq analysis identified an early stress response and disturbed cellular redox balance manifested by an increased expression of cellular signalling pathways such as oxidoreductase activity, NADPH activity, ferroptosis in response to composite materials.

**Conclusions**

The new materials achieved high degree of conversion. The amount of fluoride released was comparable to G-ænial dental composites. However, dental composite material induced signs of chemical toxicity such as impaired redox balance in oral tissues.
Endodontic Sealers Modulate the Periodontal Ligament Pro-Inflammatory Activity

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Objectives Periapical tissue inflammation control is pivotal in determining the success of endodontic treatment as this inflammation plays a significant role in apical periodontitis and bone resorption. This work was set to investigate the effect of three endodontic canal sealers: BioRoot™ Flow (BRF), Pulp Canal Sealer™ (PCS) and AH Plus® (AH) on modulating the pro-inflammatory potential of human periodontal ligament (hPDL) cells.

Methods Samples of the materials were incubated in serum-free culture media to obtain the extracts (0.2mg/ml). To simulate the endodontic sealer application on the infected tissue, hPDL cells were stimulated with lipopolysaccharides (LPS) and cultured with the materials’ extracts. Interleukin 6 (IL-6), and Tumor Necrosis Factor α (TNF-α) pro-inflammatory cytokine secretion was quantified by the enzyme-linked immunosorbent assay. The inflammatory cell recruitment sequence was investigated using a human inflammatory monocytic cell line (THP-1) and human umbilical vein endothelial cells (HUVEC). The adhesion of THP-1 to HUVEC was studied with fluorescent-labelled THP-1, their migration was investigated using Boyden chambers, and their activation into macrophage-like cells was evaluated with the cell adhesion assay.

Results Incubation of LPS-stimulated hPDL cells with the sealers’ extracts modulated the pro-inflammatory cytokines levels. While PCS significantly increased both IL-6 and TNF-α secretion, no effect was observed with AH. By contrast, BRF significantly decreased TNF-α without affecting IL-6 level. Subsequently, while the adhesion of inflammatory THP-1 cells to endothelial HUVEC monolayer was not affected by the materials’ extracts, both BRF and AH significantly decreased THP-1 migration. Finally, while all materials significantly decreased THP-1 activation, this decrease was more pronounced with BRF.

Conclusions Within the limits of this in vitro study, the endodontic sealers’ application on LPS-stimulated hPDL cells modulates their pro-inflammatory cytokine secretion and the subsequent inflammatory cell recruitment. This highlights that the endodontic sealer choice is pivotal as it modulates the hPDL cell pro-inflammatory behavior.
Rapid 3s Curing: Effect on Mechanical Properties of Bulk-Fill Composites
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Objectives This study aimed to evaluate the flexural strength (FS), modulus (FM) and microhardness (HV) of contemporary bulk-fill composites under ISO-recommended curing protocols or by varying exposure time and radiant exitance while maintaining the same density. Short-term water storage and dynamic artificial aging were compared to evaluate the reduction in material properties.

Methods Five bulk-fill composites were tested (n=10/gp): Tetric PowerFill, TPFill; Filtek One Bulk Fill Restorative, FO; QuixXfill Posterior Restorative, QX; Tetric PowerFlow, TPFlow; SDR Plus Bulk Fill Flowable, SDR). Three polymerization protocols were used: 2-mm thick specimens were polymerized according to ISO 4049 (6x20s, 1040 mW/cm\textsuperscript{2}, top and bottom), while 4-mm thick specimens were polymerized on the top surface only, either for 3x10s (1040 mW/cm\textsuperscript{2}) or 3x3s (2750 mW/cm\textsuperscript{2}). Specimens were subjected to three-point bending test and Vickers microhardness test after 24h (water, 37°C) or after 21-day (water, 37°C)+10,000 thermal cycles (5-55°C). Data were analyzed by three-way ANOVA and Tukey post-hoc (α=0.05).

Results Rapid 3s curing in 4-mm specimens produced equivalent FS (133±11MPa vs. 133±17MPa) and higher HV (61.6±3.1 vs. 49.1±1.4) to ISO-recommended curing at 2-mm depth in TPFill, a material with addition-fragmentation chain transfer (AFC) polymerization. Non-AFC materials: TPFlow, SDR, and QX after 24h also reached the same FS. Thermocycling reduced the FS in the 3s-curing group by 10-15% for all materials except TPFlow (118±7MPa vs. 119±4MPa) and reduced the HV by 4-27%. FM decreased in both 3s- and 10s-curing groups compared to ISO, especially in high-viscosity materials. FO had the highest FS (201±14MPa-175±24MPa) and QX the highest FM (13.6±1.2GPa-8.8±0.8GPa) regardless of the curing and aging regime.

Conclusions Rapid 3s curing had a strong material-dependent effect on the micro- and macro-mechanical properties of tested bulk-fill composites. While rapid curing did not influence the FS in all but one material, it induced the reduction of FM in all examined high-viscosity materials.

SWEEPS in Removing Bioceramic Sealer Remnants From Oval Root Canals
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Objectives This study compared the efficacy of two activated irrigation techniques: novel Er:YAG laser activated irrigation mode, Schock Wave Enhanced Emission Photoacoustic Streaming (SWEEPS), and passive ultrasonic irrigation (PUI) in the removal of bioceramic sealer remnants after conventional retreatment in oval root canals.

Methods Distal root canals of 36 extracted mandibular molars were instrumented with ProTaper Next (Dentsply Sirona, USA) system up to size 40/.06 and filled with bioceramic sealer (BioRoot RCS, Septodont, France) using single-cone obturation technique. After one week samples were retreated using Reciproc Blue RB40 (40/.06) file (VDW Dental, Germany) and 3% sodium hypochlorite solution (NaOCl) and, then, randomly divided into three groups (n=12) according to the final irrigation technique: 1. SWEEPS using radial laser tip; 2. PUI; 3. Conventional needle-syringe technique (NI). A total of 6 mL of 3% NaOCl was used for each canal with an activation time of 3x30 s. The samples were subjected to three micro-CT scans: after root canal filling, after root canal retreatment and after final irrigation. The volume of the filling material in each retreatment phase and the percentage reduction were calculated. The decrease in the amount of filling material after each retreatment phase was analysed using Kruskal–Wallis test. Intergroup differences were analysed using Wilcoxon Signed Ranks Test (p<0.05).

Results The results of the study showed that all tested irrigation techniques reduced statistically significant amount of the remaining filling material left after the retreatment (p<0.05). Intergroup analysis showed that there were no statistically significant differences in efficacy between all three tested groups (p>0.05).

Conclusions All tested techniques removed significant amount of bioceramic filling material during retreatment in oval root canals. All three tested groups (SWEEPS, PUI, conventional needle-syringe technique) were equally effective.
The Use of Bulk-Fill Composites Until 2019 in Oulu, Finland
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Objectives Dentists spend close to 60% of their working hours performing direct restorations. Bulk-fill composites have been available from 2003, ever since they have been reported safe and timesaving in numerous studies. There is however no data on the proportion of the bulk-fill composite containing restorations. The primary aim of this study was to determine the increase in proportion of restorations with bulk-fill composites until 2019 in Public Dental Services in City of Oulu, Finland. The secondary aim of this study was to determine the types of cases the dentists have used bulk-fill composites.

Methods The data was collected from the patient records Effica® (Tieto, Helsinki, Finland) of Public Dental Services in City of Oulu, Finland. For the study all restorations made in one day every six months from the beginning of 2011 to the end of 2019 were collected (overall from the 18 days). On each restoration operator, year of operation, tooth number, surfaces and used materials were collected. For background information the gender and age were collected for each patient. The study was performed according to the Declaration of Helsinki and the study protocol was approved by the City of Oulu Department of Healthcare (OUKA/753/07.01.04.02/2020).

Results The data comprised totally 2825 restorations made during 2011-2019. Composite was the most common restoration material used in 2507 (89.8%) restorations. Of all composite restorations, 626 (25.0%) had bulk-fill composite. All of them were on permanent teeth. The use of bulk-fill composites increased from less than 10% in 2011 to more than 40% in 2019. The increase in the use of bulk-fill composite was greatest in class II cavities.

Conclusions The results suggest that the use of bulk-fill materials has increased drastically during last decade in Public Dental Service in Oulu, Finland. Further studies are needed to investigate reasons behind that.

Dental Staff’s Experiences of Patients’ Successful Behavioral Changes in Caries Prevention
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Objectives The aim of this interview study was to obtain a deeper understanding of the dental staff’s experiences with preventive strategies relevant to successful behavioral change in patients suffering from caries disease.

Methods To investigate the aim, a qualitative research approach was selected. Nine interviews were conducted, with professional dental caregivers, including dentists and dental hygienists, at eight various public dental clinics in the southwest region of Sweden. The selection of informants was carried out strategically to obtain quality in data. The inclusion criteria were permanent employment, being professionally active within the public dental service, and having clinical experiences in caries prevention. All selected informants gave their informed consent to participate in the study. Audio-recorded interviews were transcribed and analyzed in accordance with the principles of conventional inductive content analysis.

Results The results revealed a main essence comprising ‘Dimensions of interplay during treatment’ with three underlying categories; ‘Creating an adaptive patient/therapist relation,’ ‘Implementing new behaviors,’ and ‘Managing treatment outcomes.’ The categories can be understood by subcategories of therapists and patient-management strategies. The informants experience themselves as helpers, and they have a deep belief that caries can be avoided. However, the preventive task is challenging to perform, as it involves implementing long-term individual behavioral changes in patients.

Conclusions The results of the study point to factors that could constitute keys to successful caries prevention. This is based on the creation of a treatment alliance between the patient and the therapist as well as person-centered dental care.
P129
Health-Associated Factors Against Caries in Caries Free Adults – an Interview Study
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Objectives This qualitative study aims to illuminate middle-aged patients' experiences of being and staying free from caries. The Swedish Ethical Review Authority approved the study (registration number 2020-04819).

Methods Inclusion criteria were DFT=0, age 40-50. The informants were strategically selected from the aggregated database T4, Public Dental Service, Region Västra Götaland. Invitation letters were sent and those who wanted to participate were scheduled for an interview. They provided written informed consent for interviews to be audio-recorded and data further analyzed. Up to today, six semi-structured interviews with caries-free adults, 4 men/2 women with a mean age of 44.2 ± 3.3 years have been conducted online using live meeting software (Zoom) by two of the authors (CF¹ and AA¹). Each audio-recorded interview lasted between 35 and 57 minutes, recorded on dictaphone (Zoom h2n) and was transcribed verbatim. Data was then analyzed with qualitative content analysis using an inductive approach. Interviews are ongoing and we plan to include at least another six informants.

Results The patients' descriptions of their experiences of being and staying caries-free revealed a preliminary theme 'continuity of dental care.' The informants experienced continuity of dental care as essential in staying caries-free and maintain good oral care habits.

Conclusions In summary, the preliminary results indicate the importance of continuity to dental care and that the informants are people who have been meticulous with their oral care habits both as young persons and as adults. The results will form the basis for further studies, i.e., trying to identify health factors to avoid caries throughout life.

P131
Effects of Self-Assembling Peptide P11-4, CPP-ACP, 58SS Bioactive Glass and Fluoride on Artificial Enamel Caries Lesions in Vitro
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Objectives The aim of this in vitro study is to determine and compare remineralizing efficacy of self-assembling peptide P11-4, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), 58SS bioactive glass (BG), and fluoride on artificial initial enamel caries lesions.

Methods Initial enamel caries lesions were created on samples using demineralization solution (pH 4.5, 37°C, 96 hours). Fifty permanent molars were divided into five groups with 10 samples in each group; GI: Self-assembling peptide P11-4 (Curodont), GII: CPP-ACP (GC Tooth Mousse), GIII: Experimental 58SS BG, GIV: 5% NaF varnish (Enamelast), and GV: Non-treated (Distilled water, DW). The treated samples underwent 7-day pH cycling. The remineralization potential of enamel was evaluated by Vickers hardness test (%REMH). Surface roughness (Ra) assessment was done using a profilometer. The surface topography of representative specimens from each group was inspected using a scanning electron microscope. Analyzes were performed at three stages: at baseline value, after demineralization, and after the pH cycle. Collected data were analyzed using two-way ANOVA followed by Bonferroni post hoc test at p<0.05.

Results The aim of this in vitro study is to determine and compare remineralizing efficacy of self-assembling peptide P11-4, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), 58SS bioactive glass (BG), and fluoride on artificial initial enamel caries lesions.

Conclusions It can be concluded that experimental 58SS BG use has a better remineralization effect on enamel than the other tested materials.
P133

Cannabinoids as a Natural Non-Invasive Alternative for Decay-Intervention
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Objectives Our aim was to evaluate the antibacterial effect of cannabinoid extracts on caries-inducing bacteria in vitro, and to examine its efficacy as a caries-preventive modality using a novel in vitro caries model.

Methods The lytic activity of four cannabinoid extracts against Mutans streptococci was tested in vitro, by Direct contact test. Then, hemi-mandibles dissected from euthanized healthy mice were autoclaved and subjected to caries-promoting conditions in vitro. After 5 days the mice teeth were evaluated. Evaluation included caries scoring using hemi-sectioned first molars, photographic depiction and microCT analysis.

Results Two cannabinoid extracts have shown to inhibit the bacterial growth and prevent caries, while other two extracts have shown to enable bacterial growth, with significant demineralization of the enamel and dentin.

Conclusions Our results indicate that certain cannabinoid extracts can inhibit S. mutans bacterial growth and prevent carious lesions development. The present finding suggests a new and promising idea of using cannabinoid extracts as a novel modality to combat caries-inducing bacteria.

P134

Impact of Different Toothpaste-Formulations on Salivary Fluoride and Calcium Concentrations
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Objectives There is a general consensus regarding the positive effects of daily usage of fluoridated toothpaste for caries prevention. Both choice of product and individual behavior are today considered as important factors for an optimal effect. The aim of the current study was, in a randomized double-blind study design, to in vivo compare the dissolved fluoride (F) and calcium (Ca) concentration of four different fluoride toothpaste formulations in saliva.

Methods Twenty subjects used in a standardized manner toothpaste products containing 1) NaF, 2) AmF, 3) SnF2 – all with 1450 ppm F, and 4) fluoride in a bioactive glass formulation with 530 ppm F. Approximal fluid and whole saliva were collected before and up to 60 min after brushing for analyses of fluoride content. Unstimulated saliva was also collected for assessment of calcium content. In addition, pH of the products was analyzed.

Results The F concentration at 2 min differed significantly between the bioactive glass and all other three toothpaste formulations (p<0.05). When expressed as area under the curve for the time period 0-60 min (AUC0-60), the AmF- and NaF-formulations resulted in the highest total F concentration, which differed significantly in comparison to the bioactive glass formulation (p<0.001). For calcium, a higher salivary concentration was found for NaF and AmF and the bioactive glass formulation in comparison to the SnF2 formulation during the first minutes after brushing (p<0.05). The fluoride and calcium concentrations seem to be related to the time and pH. The highest pH was found for the bioactive glass formulation followed by NaF, AmF and SnF2.

Conclusions All four fluoride toothpaste formulations indicate caries preventive properties, but with a favour for the NaF- and AmF-formulations. The relation between fluoride and calcium concentrations seems to be highly dependent on the pH of the product.
Antimicrobial Activity of Topical Potassium Iodide and Silver Solutions

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Objectives Commercially available topical silver compound solutions such as Riva Star (SDF) and Riva Star Aqua (RSA) (both SDI, Australia) contain 3.16M silver diamine fluoride and 3.16M silver fluoride, respectively. These products are available as ‘two-step applications’ which involve the application of potassium iodide solution (provided by the manufacturer) after the silver compounds to minimise the black staining produced by silver. These topical silver compounds are used clinically for their bactericidal and cariostatic effects. The aim was to investigate the antimicrobial properties of SDF and RSA, with and without KI.

Methods Minimum bactericidal concentrations (MBC) were determined for *Streptococcus mutans* for the following test solutions (at a concentration of 3.16M): Silver Diammine Fluoride (SDF – Step1), Silver Fluoride (RSA – Step 1), Silver Diammine Fluoride + Potassium Iodide (SDF – Step 1&2), Silver Fluoride+Potassium Iodide (RSA – Step 1&2, SDI). Control solutions at the same concentration were: potassium iodide (Step2, SDI), silver nitrate (Chem Cruz, California), potassium fluoride (Sigma-Aldrich, USA), silver nitrate + potassium fluoride (Sigma-Aldrich, Chem Cruz). MBC assays were carried out in a 96-well plate format with *S. mutans* bacterial suspensions at 0.1 OD<sub>600</sub> under aerobic conditions.

Results Minimum bactericidal concentrations for the tested solutions are given in Table 1. KI very significantly reduces the bactericidal efficacy of both SDF and RSA, by (approximately 10<sup>6</sup>).

Conclusions SDF and RSA have a strong antibacterial effect. The addition of KI to SDF and RSA reduces their antibacterial effect considerably. Although the two-step application of KI after SDF and RSA may reduce the black staining of the carious lesion, it significantly reduces the cariostatic bactericidal action.

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Minimal bactericidal concentration of different solutions to Minimal bactericidal concentration of different therapeutic solutions to <em>S. mutans</em></th>
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<tbody>
<tr>
<td>SDF</td>
<td>0.001mM</td>
</tr>
<tr>
<td>RSA</td>
<td>0.005mM</td>
</tr>
<tr>
<td>SDF+KI</td>
<td>350mM</td>
</tr>
<tr>
<td>RSA+KI</td>
<td>350mM</td>
</tr>
<tr>
<td>KI</td>
<td>1060mM</td>
</tr>
<tr>
<td>AgNO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1.4mM</td>
</tr>
<tr>
<td>AgNO&lt;sub&gt;3&lt;/sub&gt;+KF</td>
<td>0.001mM</td>
</tr>
<tr>
<td>KF</td>
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</tbody>
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The Influence of Sucrose on a Multi-Kingdom Cariogenic Biofilm
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Objectives To develop a polymicrobial biofilm and assess the impact of C. albicans and sucrose on its cariogenic potential.
Methods A selection of biofilm communities were prepared (4 bacteria Streptococcus oralis, Actinomyces oris, Actinomyces viscosus) (4), 5 bacteria (4 + Lactobacillus acidophilus) (5L), 5 bacteria with L. acidophilus added 3rd day (5L3)) with/without C. albicans grown on hydroxyapatite discs with artificial saliva (AS) ± 0.2 % sucrose. AS was changed daily days 1-5, then starved. Biomass, metabolic activity and microbial composition were measured using crystal violet, XTT assays and qPCR respectively. To determine cariogenic activity, 5L3 biofilms ± C. albicans were grown on polished human tooth enamel and characterized by non-contact profilometry to measure surface roughness (Sa), Raman spectroscopy and Knoop microhardness (KHN).
Results Sucrose influenced the biofilm species composition, with S. mutans and C. albicans being more abundant in sucrose-mediated biofilms than in non-sucrose-mediated biofilms. S. mutans was reduced in Candida-containing versus Candida-free biofilms in the absence of sucrose. The abundance of S. oralis was higher in Candida-containing biofilms than in Candida-free biofilms and higher in sucrose-containing medium. There was an increase in Sa of enamel in all biofilms and mediums. The Candida-free 5L3 community with sucrose, on the other hand, had considerably increased Sa. Raman spectroscopy indicated a loss of mineral content, with the greatest loss in sucrose-containing biofilms. KHN was decreased in sucrose-containing biofilms. Furthermore, Candida-free biofilms with no sucrose had lower KHN than Candida-containing biofilms. All biofilms medium had a lower pH on day 10 than on day 1.
Conclusions Within 10 days, the polymicrobial biofilms produced in this study developed cariogenic communities on enamel surfaces. Changes in biofilm species composition are influenced by multi-kingdom biofilms and sucrose, which has an impact on biofilm cariogenicity. C. albicans reduces demineralization, whereas sucrose enhances it.

EPS-Rich Biofilm Does not Modify Chlorhexidine Antimicrobial Effect
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Objectives Extracellular polysaccharides (EPS) in dental biofilm matrix may interfere on the antimicrobial effect of chlorhexidine. Therefore, we investigated the effect of a 0.12% chlorhexidine solution on cell viability in two in situ formed biofilms: one rich in EPS (EPS+) and other not (EPS-).
Methods A double-blind, randomized, split-mouth study was conducted in 2 phases of 21 days. Six volunteers used a palatal appliance containing 3 bovine enamel blocks. Three blocks were exposed 8x/day to a 20% sucrose solution (EPS+), and the other two to a 10.5% glucose + 10.5% fructose solution (EPS-) during the entire experiment. After the first week of biofilm accumulation, volunteers started to rinse 2x/day a solution of 0.9% sodium chloride (negative control - NC) or 0.12% chlorhexidine (CHX) for 7 days. The rinsing was stopped, and the volunteers kept using the device for more 7 days. Four groups were evaluated: (i) EPS-/NC; (ii) EPS+/NC; (iii) EPS/CHX and (iv) EPS+/CHX. Biofilm and its respective block were collected on the 7th day (baseline), on the 14th day (after treatment) and on the 21st day (after treatment interruption). Biofilm was resuspended in 0.9% NaCl, plated on blood agar, incubated at 37oC, 10% CO2 and the CFU counted. Three-way ANOVA was performed considering biofilm, treatment and time as factors (α=5%).
Results At baseline, CFU counts (x10⁶ CFU/mg wet biofilm) were similar among the groups (p>0.05). After treatment, the counts for EPS+ and EPS- were, respectively, for negative control groups 9.6 ± 2.6 and 7.3 ± 3.4, whereas for CHX groups were 2.9 ± 5.1 and 2.0 ± 2.4. Statistical analysis showed differences only between treatments (p<0.01), by not for biofilm type. After interruption, the counts were similar among all the four groups (p>0.05).
Conclusions An EPS-rich biofilm does not reduce chlorhexidine antimicrobial effect.
Cell viability (CFU/mg biofilm wet weight) by biofilm type, treatment and time
Analyses of Herbal Toothpaste's Antimicrobial Efficacy: in Vitro Study
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Objectives
The aim of this in-vitro study is to evaluate the antibacterial efficacy of different toothpastes on Streptococcus mutans, Lactobacillus acidophilus, Actinomyces viscosus, Candida albicans, Staphylococcus aureus and Escherichia coli.

Methods
Three conventional toothpastes:
1. Signal Expert Protection (1450 ppm NaF) (Unilever, Bulgaria)
2. Dentasave Klorhex Intensive Care (0.2 % chlorhexidine digluconate) (Drogsan, Turkey)
3. Dentasave Klorhex Daily Care (0.05 % chlorhexidine digluconate) (Drogsan, Turkey)

Two herbal toothpastes:
1. Gumgumix (ginger and honey, Beka Ilaç, Turkey)
2. Colgate Hemp Seed Oil (Colgate, Palmolive, USA)

Six herbal experimental toothpastes (SPC Kozmetik, Turkey)
1. 5% hemp oil + 5% hemp extract
2. 5% hemp oil + extract+Ag nanoparticle
3. 5% hemp oil + extract+Zn nanoparticle
4. 5% hemp oil + extract+Cu nanoparticle
5. 5% hemp oil + extract+Fe nanoparticle
6. 5% hemp oil + extract+Ag+Zn+Cu+Fe nanoparticle

And one base experimental toothpaste were tested for antimicrobial activity by agar-well diffusion method. Streptococcus mutans, Lactobacillus acidophilus, Actinomyces viscosus, Staphylococcus aureus, Escherichia coli and Candida albicans strains were prepared at 10⁸ CFU/mL, then spread on agar media. Wells with 6 mm diameter were punched and samples were transferred. Paper discs containing 0.2% chlorhexidine digluconate were used as positive control. After incubation at 37°C for 24-48 hours, the inhibition zones formed around the wells were measured.

Results
It was observed that Klorhex Intensive Care and one experimental toothpaste (containing Ag nanoparticle, hemp oil, hemp extract) are effective against all microorganisms used in the study. The ginger-containing herbal toothpaste didn’t show antimicrobial activity against the microorganisms. Signal Expert Protection, Colgate Hemp Seed Oil and Klorhex Daily Care toothpastes are effective against all microorganisms in the study except E. Coli.

Conclusions
While the addition of hemp oil and extract increased antibacterial activity in experimental toothpastes, Gumgumix toothpaste containing ginger and honey didn’t have an antibacterial effect.
P142
Treatment of an Endo-Periodontal Lesion in an Uncontrolled Diabetic Patient
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Objectives
The relationship between periodontal disease and diabetes is known. Our aims are local (reduction of plaque levels compatible with periodontal health, reduction of gingival inflammation, reduction of probing depths, resolution of bone lesions) and general (diabetes control).

Methods
35-year-old female patient with type I diabetes (glycated hemoglobin = 8.4%) treated with insulin. She had stage III grade C periodontitis associated with a grade 3 endo-periodontal lesion without root damage out of 26.

After modification of periodontal hygiene method, a non-surgical periodontal therapy was undertaken: supragingival professional mechanical plaque removal, subgingival instrumentation of pockets ≥4 mm under systemically administered antibiotics (Amoxicilline and Metronidazole). Endodontic treatment of 26 was performed during non-surgical periodontal therapy. Reevaluation at 12 weeks showed probing depths ≤5mm except on 26 (PPD ≥ 9mm). In addition, tooth 26 had a mesial and distal class II furcation involvement. Surgical periodontal treatment of 26 by periodontal regenerative therapy enamel matrix derived combined with bone derived graft was then performed. After the Periodontal Risk Assessment, a periodontal follow-up was set up every three months.

Results
At 9 months post-surgery, the plaque index was 18% and the bleeding on probing 10%, all probing depths were ≤5mm. Radiographic control of 26 shows healing. The patient’s glycated hemoglobin is now 7.6%.

Conclusions
Treatment of periodontal disease and its lesions can be achieved with periodontal and systemic benefit in a patient with poor initial plaque control and uncontrolled diabetes.
Recalcitrant Sinusitis of Odontogenic Origin

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Objectives To report a case of recalcitrant sinusitis development originated from peri-implantitis, discuss the challenges of interdisciplinary management and raise awareness of careful implant placement and possible complications.

Methods In 2019, a 39-year-old woman underwent an implant placement in posterior maxilla. Postoperatively the patient developed persistent symptoms of trigeminal neuralgia, which resulted in implant removal. During the examination, an oroantral fistula formation was observed. The patient was referred to an otorhinolaryngologist due to developed purulent nasal discharge and intensified pain. Clinical examination revealed purulent discharge under the middle concha and sealed oroantral fistula. Dilatation of anatomical sinus opening and irrigation of the sinus was performed. Antibacterial treatment was prescribed. However, the patient returned to the clinic with recurrent symptoms. Later on, she underwent type II endoscopic maxillary antrostomy in June 2020. Yet, the symptoms have recurred. During paranasal sinus CT, MRI examinations only minimal mucosal changes were observed on the maxillary sinus floor. Several dentists inspected the patient, but no dental pathology was detected. In February 2021, revision endoscopic maxillary antrostomy was performed, nevertheless sinusitis has recurred after one month. Due to failed treatment, the patient underwent a modified endoscopic medial maxillectomy. During the procedure, medial maxillary wall was removed, enabling better irrigation, aeration and drainage of the sinus.

Results A modified endoscopic medial maxillectomy was performed successfully, without any postoperative complications. During follow-up examinations at 3 and 6 months, the patient showed a complete remission of the sinusitis.

Conclusions Since implant-related infection can spread to maxillary sinuses, periorbital area or cavernous sinus, interdisciplinary work is significant between oral surgeons and otorhinolaryngologists when treating such patients. Eliminating the source of infection in odontogenic sinusitis is the most important factor in preventing the disease from recurring or exacerbating. When conservative and conventional surgical treatment fails, modified endoscopic medial maxillectomy is the preferable alternative choice of treatment.
Multidisciplinary Management of Anterior Polydiastema: a Case Report

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Objectives Anterior diastema may compromise the harmony of a patient's smile. Consideration of etiologic factors, previous gingival aesthetics, and individual treatment planning are essential in the proper management of anterior diastema. The aim of this case is the aesthetic rehabilitation of the polydiastema with direct composite-resin restoration (DCRR) and ensure aesthetic harmony with the gingival tissue.

Methods 28-year-old female patient was referred to our clinic complaining of her smile. Following clinical and radiological evaluations, gingivectomy, bleaching and DCRR were planned for the rehabilitation of teeth #13-23. Once the teeth dimensions were recorded, gingivectomy was performed via a diode laser (810nm wavelength, 3W power, 400µ fiber-tip in contact mode, Cheese) to obtain the proper 75-80% width/length ratio. 3 weeks following gingivectomy, office-bleaching was applied for 4x15min (%40 HP Opalescence Boost, Ultradent). 10 days after bleaching, polydiastema closure was performed with DCRR (Ceram.x SphereTEC one A2, Dentsply). Under rubber-dam isolation, Optibond Universal (Kerr) was applied with total-etch technique (Vococid, Voco) without preparation. Transparent matrixes (CoForm, Directa) were placed for restoring teeth. Composite-resin and the adhesive were polymerized with Valo (Ultradent). Finishing and polishing procedure was performed with discs (Soflex), interdental strips (Epitex, GC) and spiral discs (DiaComp-Twist, Eve). The patient was followed-up on the first week, first and 6th months, in accordance with the FDI criteria.

Results Treatment plan of the case including gingivectomy, bleaching and direct composite-resin restoration was scored as 1 according to the FDI criteria in terms of functional, biological and aesthetic properties.

Conclusions To achieve an aesthetic harmony, often a multidisciplinary approach is necessary. In the present case, the cooperation in between periodontologists and restorative dentistry specialists helped solve the complexity of the case while respecting biological, functional and aesthetic principles. Gingivectomy, bleaching and DCRR are conservative treatment options for aesthetic rehabilitations of polidiastema cases.

The Third Molar Autotransplantation: 4 Case Series

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Objectives Autotransplantation; it can simply be defined as extracting the impacted, semi-impacted or erupted teeth and transplanting the recipient socket. Although implants provide satisfactory comfort with long-term success in tooth loss, autotransplantation may be preferred as a treatment option in some cases such as tooth loss combined with a suitable donor tooth.

Methods Case report
A total of 4 patients who had wisdom teeth, 2 male and 2 female, aged between 19-27, applied to our clinic for tooth extraction. After extraction of irreparable teeth, wisdom teeth were extracted in the same session and placed in the recipient socket. Two days later, the teeth on the side were splinted to provide stabilization. Canal treatments were performed within 3 weeks

Results All cases were successful after a one year follow up period. Autotransplantation is a biologically advantageous treatment option in the patient with the correct indication for tooth loss. It is economically affordable and one of the fastest treatment methods for tooth deficiency. Considering prognostic factors, the success rate is quite high.

Conclusions Tooth autotransplantation can be considered as an alternative approach in oral rehabilitation for some clinical situations (especially in young patients). It inducts bone formation, and re-establishment of a normal alveolar process. The procedure needs more consideration and future clinical studies in order to obtain predictable long-term results.

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Objectives
During the last 15 years, molar incisor hypomineralization (MIH) has been gaining attention in pediatric dentistry. MIH clinically presents in incisors and the first permanent molars (FPMs) as demarcated enamel opacities of a different color. Its severity differs not only between patients but also within the mouths of the patients, and its appearance can be asymmetrical.

Due to soft and porous enamel, more severely affected teeth can undergo post-eruptive breakdown of hard tissue.

Methods
The therapy of MIH teeth using a complete composite restoration.

Results
Treatment consisted of the immediate pain elimination, restoration and protection against further fractures and discussion of oral hygiene and prophylaxis for a MIH teeth.

Conclusions
Easy stepwise treatment for MIH teeth is presented.

The Use of Direct Composites in Repair of Broken Teeth

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Objectives
In this case, it was aimed to restore the teeth of 17 years old female patient who applied to our clinic with the complaint of the aesthetic appearance of her teeth, which were broken as a result of an accident, by using direct composite resin.

Methods
Enamel dentin fracture was detected in the tooth in which the pulp did not exposure as a result of trauma and no symptom was observed. After the first intraoral photographs, the maxilla measurement was taken with alginate impression material for a silicone key. Then model was obtained with dental plaster. With that plaster “Wax up” model was obtained with modeling wax. Afterwards, Silicone Index was prepared with C-type silicone (Zhermack).

When the patient came; the composite color was decided and rubber-dam isolation was applied. Minimal beveling was done to the enamel edges with a diamond bur. For 30 seconds, 37% phosphoric acid gel (Ruby Etch) was applied to the broken surfaces of teeth, than washed with water and dried. Optibond’s ™ XTR (Kerr) adhesive system was applied and using by silicone index the palatal wall was formed. Later on Ceram X SphereTEC one (Dentsply Inc.) was placed on teeth using the composite layering technique and LED light was applied for 40 seconds from all directions. Finishing was done with yellow banded burs, after than polishing was carried out using discs (Ruby Platon) and tires.

Results
2 years later; the patient who came to the control, no fracture was found in the restoration and just polishing was applied to the discoloration.

Conclusions
Nowadays, broken teeth which restored with direct composite resin are considered successful for the patient and the physician. Reasons for this success are decrease in the number of sessions, these restorations are polishable, repairable and tooth-colored. Consequently tendency of the direct composite restorations are increasing gradually.
Treatment of Tongue Arterio-Venous Malformation (AVM) With Dye-Laser: 36-Month Follow-Up
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Objectives Arterio-Venous Malformations (AVMs) are abnormal communications between blood vessels that bypass the normal capillary bed. To avoid the invasiveness of the gold standard surgical exeresis, dye-laser has been suggested to coagulate both capillaries and red vascular malformations, without involving the superficial epithelial layers.

Methods A 53-year-old man with a good systemic health and difficulties in chewing and phonation presented a voluminous bluish-red nodular neoformation covered by intact mucosa, with hard-elastic consistency and no pain on palpation, on the left hemitongue. Tomographic ultrasound imaging (TUI), Color/Power Doppler and 3D Angio showed a capsulated neoformation. The lesion was diagnosed as AVM and classified as stage 2 according to Schobinger et al. (1996). The patient underwent two applications within 2 months of rhodamine dye-laser with the following parameters: fluence at 12 J/cm², handpiece with 6 mm spot, single pulse with repetition up to 1.0 Hz and pulse duration of 3.0 ms.

Results One month after the first application, the lesion was reduced in volume but appeared stable in colour; thus, a second application was provided to improve the same parameters. At 24 months follow-up, the lesion showed a size reduction with non-anarchic vessels arborization, however, a fibrotic structure persisted, requiring a peri-structural injection of cortisone. Thirty-six months after treatment the obtained outcomes remained clinically stable.

Conclusions With the limitations of the present case report, the application of dye-laser appeared to be successful in treating AVMs. Further studies are needed to study in depth the efficacy and morbidity of dye-laser in the treatment of AVMs compared to traditional therapeutic options.
P152
Influencing Factors on Students’ Performance Using a Preclinical Endodontic Analytic Rubric
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Objectives To determine the most influential evaluated factors in students’ grades during the endodontic preclinical course using an analytic rubric

Methods The grades obtained from 252 root canal treatments performed in extracted human molars by fourth-year students during their endodontic preclinical course were evaluated by an analytic rubric. The following treatment-related factors were examined to determine their impact on students’ overall scores: anatomical difficulty (high/moderate/low), type of tooth (upper/lower molars) and 3 different endodontic shaping techniques (hand-file motion, continuous rotary motion with Protaper Gold files, and reciprocating motion with Reciproc Blue files). Data were analyzed by three-way ANOVA and Tukey tests (p<0.05).

Results The variables anatomical difficulty (p=0.001) and the interaction between this factor, shaping endodontic techniques and type of tooth (p=0.008) were statistically significant. The endodontic shaping techniques, regardless the degree of difficulty, had no influence on the final scores of endodontic treatments in upper molars. In lower molars treatments with low and high anatomical difficulty, the resultant scores were not affected by the shaping procedure. However, in those with a moderate anatomical difficulty, statistically lower scores were achieved with the hand-file shaping technique.

Conclusions The scores achieved by the students when using continuous rotary and reciprocating motion shaping techniques were not influenced by anatomical difficulty nor type of tooth.

P154
Web-Based Program Reduced Dental Anxiety in Lithuanian and Norwegian Adults
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Objectives To assess the short-term effectiveness of the web-based program to reduce dental anxiety among adults in Lithuania and Norway.

Methods Pre-test and post-test design was used. For the web-based programs, tailor-made websites were developed in Lithuania and Norway. Based on the sample size calculation, 34 participants in each country were needed. Volunteers having self-reported dental anxiety were invited to participate and those who signed informed consent were included in this study. Data was collected using an online questionnaire, which included participants’ sociodemographic data and their awareness of family dental anxiety history. Dental anxiety levels measured by the Modified Dental Anxiety Scale (MDAS) were recorded at the baseline and after 2 weeks. Chi-square and Mann-Whitney U tests were used to compare participants’ characteristics between countries. Wilcoxon Signed Rank test was used to compare median baseline and post-program MDAS scores.

Results The program was completed by 34 participants in Lithuania and 35 participants in Norway. In both countries, the majority of participants were females residing in urban areas. In Norway, the participants were younger, and a higher proportion of them had secondary school education (vs bachelor’s degree and more), while a higher proportion of participants in Lithuania did not know family dental anxiety history. Baseline median MDAS scores were not statistically significantly different between countries. In Lithuania, the median post-program MDAS score (9.5, interquartile range (IQR) 5.25) decreased compared to the median baseline MDAS score (14.5, IQR 8) (Z value=-4.246, p<0.001). The same was true in Norway; the median post-program MDAS score was lower (12, IQR 9) compared to the median baseline MDAS score (15, IQR 7) (Z value=-3.818, p<0.001).

Conclusions After 2 weeks, the participants in both countries reported reduced levels of dental anxiety. Studies with more controlled designs and longer-term outcomes are needed to validate results of this study.
45kHz Ultrasound Influences Endothelial Cell Growth and Gene Expression

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**Objectives** Angiogenesis is required in hard and soft tissue repair and involves the differentiation, migration and proliferation of endothelial cells to develop new blood vessels. The majority of the studies investigating the effect of ultrasound on angiogenesis involve the use of ultrasound (US) in the megahertz (MHz) frequency range. Little is known about the effects of kHz frequencies, which are used in ultrasonic surgical devices. The present study aimed to explore the effect of kHz ultrasound on endothelial cell behaviour.

**Methods** Human umbilical vein endothelial cells (HUVECs, Promocell, Germany) cultured in supplemented endothelial growth medium (PromoCell, Germany) were seeded in a 35mm Petri dish. A DuoSon US therapy device (SRA Developments Ltd, Ashburton, UK) treated cells with 45kHz continuous wave US for 5 mins at varying intensities (10, 25 & 75mW/cm²). Effects on cell number and viability were analysed 24h and 72h post-treatment using trypan blue viability analysis. Semi-quantitative reverse transcription polymerase chain reaction (sqRT PCR) identified expression levels of endothelial cell marker genes and angiogenesis-related genes including vascular endothelial growth factor A (VEGFA) and its corresponding receptor (VEGFR2).

**Results** Cells counted 24h after US treatment showed a modest increase in cell number when compared with controls. After 72h, a statistically significant increase in cell number was seen in all US-treated groups. Ultrasonic intensities of 25mW/cm² showed the greatest increase in cell number, 27% higher than controls. Cell viability was unaffected after all US treatment. VEGFA expression was elevated in all ultrasonic treated groups after 72h; 25mW/cm² resulted in the highest expression increase. VEGFR2 expression increased after 25 & 75mW/cm² treatment compared with controls and 10mw/cm² exposure.

**Conclusions** 45kHz continuous wave US affected endothelial cell numbers and gene expression in a dose-dependent manner. Therefore the use of low frequency ultrasonic surgical devices may influence angiogenesis and subsequent wound healing during and after hard and soft tissue cutting procedures.

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P156

Structural Characterization of Human Dental Pulp Tissue Using Multiphoton Microscopy

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**Objectives** To perform a structural characterization of dental pulp tissue using multiphoton microscopy and to compare this to classical histological coloration.

**Methods** Sample preparation: Freshly extracted teeth with healthy pulp were used in this study. All teeth were fixed in PFA 4% for 24 hours, rinsed in PBS and decalcified. The samples were then embedded in paraffin. The paraffin blocks were cut with a Microm HM340E microtome with Niagara system to obtain 3μm thick sections. The samples were then dewaxed with xylene, rehydrated and stained with Hematoxilin/eosin and Masson’s trichrome. Multiphoton Microscopy (MPM) images were recorded using a custom-built multiphoton microscope based on a SliceScope microscope (MPSS-1000P, Scientifica) upright microscope. MPM images were obtained recording the non-linear emission spectra after laser scanning of samples. Two-photon fluorescence (2-PF) signal was used to image autofluorescent structures. Second harmonic generation signal (SHG) was performed to image collagen. The recorded images were then compared to the histological stained sections observed under an optical microscope.

**Results** At the dentin/pulp junction, we observed the dentin, which emitted an important SHG signal due to the abundance of type I collagen fibers. The palisade of odontoblasts and odontoblast extensions (2PF) were imaged towards the dentinal tubules. We imaged blood vessels due to the 2-PF signal of blood cells. A fibrous structure representing the extracellular matrix emitted a SHG signal, showing the presence of type I collagen fiber. At the apical 1/3 of the root fibers were observed at the periodontal ligament emitting both in SHG and 2PF, coming in contact to the pulp. The MPM images were then compared to the histological stained samples.

**Conclusions** Multiphoton microscopy is a convenient label-free minimally invasive technique to image live, vascularized and dynamic tissue such as the pulp. It provides valuable additional information with respect to histological sections observed under an optical microscope.
The Isolation and Characterisation of Ovine Dental Pulp Stromal Cells

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Objectives

Dental pulp stromal/stem cells (DPSCs) are an attractive cell source to regenerate injured tissues. In particular, they represent a gold standard for neural-crest-derived tissue reconstruction and can be used in low-risk autologous therapeutic strategies. Sheep have been proposed as an animal model for a wide range of applications in biomedical research, such as tendon, cartilage, bone and nerve repair, that cannot be mimicked in smaller animals. Therefore, the study aimed to characterise ovine dental pulp stromal/stem cells (oDPSCs).

Methods

Cone beam CT scans were used to select the most suitable teeth for pulp extraction in adult sheep (50–85 kg). Selected teeth were extracted in sedated animals by applying a light rotational force. A sterile number 20 Hedström endodontic file was used to access the root canal space through the apex to extract the pulp tissue. oDPSCs were then isolated from extracted pulp tissue according to the explant method. Next, oDPSCs were characterised by measuring the expression of the stem cell markers CD44, CD90, and CD146 via immunocytochemistry. Furthermore, we tested their multilineage differentiation potential.

Results

Based on cone beam CT scans, lower anterior central incisors were extracted because their morphology seemed similar to human incisors, with an open apex and large pulp cavity. We successfully isolated oDPSCs, which were plastic adherent, expanded easily in culture and had a fibroblast-like morphology. We demonstrated that these cells express the mesenchymal markers CD44, CD146 and CD90 at the protein level. In addition, we confirmed their mesenchymal properties through trilineage differentiation towards chondrocytes via increased aggrecan expression, and towards osteocytes via positive Alizarin-Red staining and towards adipocytes.

Conclusions

We successfully isolated DPSCs from ovine teeth and showed their stemness. These cells can be used in the future for large animal tissue regeneration studies.
Morphometric Study of Incisors Dental Pulp of Ovine Preclinical Model
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Objectives In a pilot study, we showed that dental pulp stromal/stem cells can be isolated from lower incisors in the sheep, a commonly used preclinical model in regeneration research. This study aimed to characterize and quantify dental pulp in ovine incisors to refine collection of dental pulp.

Methods Lower incisors (sheep have 4 lower incisors and no upper incisors) of 2-year-old (N=3), 3-year-old (N=9), 4-year-old (N=3), and 6-year-old (N=5) sheep were scanned by Computed Tomography (CT) (120kV, 269mAs, 0.67-mm thick slices). Dental pulp volume was measured after reconstruction. A statistical mixed linear model was used for analysis. Sections of full incisors and of isolated dental pulp were stained with Haematoxylin Eosin Safran.

Results Histology revealed that ovine incisors and dental pulp had a tissue organization similar to that in man. The more lateral incisors (second intermediate and corner) were still deciduous in 2-year-old sheep and were not included in analysis. Dental pulp volume, at CT, decreased with age, and location from the more central (pincer, I1) to the more lateral incisors (corner, I4). Mean dental pulp volume ranged from 36.7+/−6mm^3 to 19.6+/−6mm^3 in 3 year-old sheep, and from 19.4+/−5mm^3 to 11.5+/−4mm^3 in 6-year-old sheep. Dental pulp volume was significantly different between young (2- or 3-year-old) and older sheep (4- or 6-year-old) (P= from 0.008 to 0.0001). No significant statistical differences were found between I1 and the first intermediate (I2) teeth. The pulp volume of I1 was significantly higher than that of the second intermediate incisor (I3) (P<0.03). Mean pulp volume was 34.0+/−7.1mm^3 for I1 and 40.2+/−16mm^3 for I2.

Conclusions Central incisors (pincer and first intermediate) of 2- and 3-year old sheep should be preferentially selected in order to collect a maximal amount of dental pulp for further regenerative research.

Dental pulp volume measured by CT scan (mean +/−SD in mm^3)

<table>
<thead>
<tr>
<th>Age / Teeth</th>
<th>I1 (pincer)</th>
<th>I2 (first intermediate teeth)</th>
<th>I3 (second intermediate teeth)</th>
<th>I4 (corner)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-year-old sheep</td>
<td>34.05 +/- 7.11</td>
<td>40.18 +/- 16.15</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>4-year-old sheep</td>
<td>23.6 +/- 5.19</td>
<td>20.07 +/- 4.62</td>
<td>16.93 +/- 3.06</td>
<td>11.26 +/- 2.17</td>
</tr>
<tr>
<td>6-year-old sheep</td>
<td>19.35 +/- 4.71</td>
<td>18.06 +/- 3.28</td>
<td>14.61 +/- 3.07</td>
<td>11.51 +/- 3.69</td>
</tr>
</tbody>
</table>
Vital Pulp Treatment (VPT) Application – Commonality Across Clinician Groups?

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Objectives To explore the application of VPT across undergraduate clinical teachers (UGTs) and dental foundation/vocational training groups (DFGs) in managing deep caries.

Methods A cross sectional study was conducted across the United Kingdom (UK) (n=16) and Republic of Ireland (ROI) (n=2) dental schools and the UK DFGs (n=10) including Foundation Dentists and Educational Supervisors. Participants completed an electronic questionnaire selecting responses to clinical vignettes and identifying any factors influencing those decisions. Descriptive statistics was used to analyse data.

Results 243 valid responses were received (n=85 UGTs, n=158 DFGs). 17% of participants commenced root canal treatment (RCT) if pulp was exposed during cavity preparation and 24% used sodium hypochlorite in pulpal haemostatic protocols. In isolated irreversibly inflamed vital pulps (IRP), 15% of participants would carry out direct pulp cap (DPC), 26% pulpotomy and 59% RCT. Clinician role was associated with decision-making. DFGs more often carried out RCT than UGTs (65% v 47%), p=0.01 in vital IRP cases. 89% UGTs reported using rubber dam all or most of the time during VPT dropping to 67% for DFGs, p<0.001. 6% UGTs commenced RCT of an exposed vital pulp compared with 23% DFGs p=0.028. Use of hydraulic calcium silicate cements (CSCs) was seen across both groups in DPC but was significantly higher in UGTs p<0.001. 81% UGTs recommend VPT to students with caries associated with decision making. DFGs more often made recommendations to students. Use of hydraulic calcium silicate cements (CSCs) was seen across both groups in DPC but was significantly higher in UGTs p<0.001. 81% UGTs recommend VPT to students.

Conclusions Despite offering a less technically demanding alternative to RCT, VPT application is at odds with current recommendations. Teachers appear consistent with their decision-making and recommendations, but this does not always translate into primary care practice. Better understanding of facilitators and barriers to biologically-based treatments is required if benefits are to be realised.

Multicomponent Peptide Hydrogel Culture of DPSCs to Assess Anti-Inflammatory Properties

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Objectives Peptide hydrogels have emerged as promising self-assembling scaffolds for regenerative therapies. Multicomponent peptide hydrogels functionalised with the adhesion motif Arg-Gly-Asp (RGD) have shown promise in our previous studies. Further work is now required to support our understanding of the anti-inflammatory nature of these hydrogels, to help advance their translation for therapeutic use.

The objectives of this work were to show that Stro-1+ve human dental pulp stem cells (DPSCs) could be encapsulated in multicomponent peptide hydrogels containing 40% or 50% RGD peptides and to determine levels of the pro-inflammatory cytokine interleukin 8 (IL-8) in conditioned media following hydrogel culture for 7 days.

Methods Stro-1+ve cells were encapsulated in multicomponent peptide hydrogels containing either 40% or 50% RGD peptides. Cells encapsulated in hydroxypropyl methylcellulose (HPMC) served as hydrogel (3D) controls. Cells grown in conventional 2D culture served as additional controls. Cells were encapsulated in hydrogels, and after 24 hours were stained with 4',6-diamidino-2-phenylindole (DAPI) and phalloidin. Images were obtained by confocal microscopy. In additional experiments, cells were cultured for 7 days and following this time the conditioned media was collected and analysed for IL-8 levels by ELISA.

Results Stro-1+ve cells were successfully encapsulated in all hydrogels studied, as shown by confocal microscopy. Following 7 days in culture, ELISA results indicated that conditioned media samples from multicomponent hydrogels, functionalised with either 40% or 50% RGD, contained significantly less IL-8 compared to conditioned media from HPMC hydrogels or 2D controls.

Conclusions Multicomponent peptide hydrogels functionalised with 40% or 50% RGD show promise for use as 3D culture platforms for Stro-1+ve DPSCs. Future applications for these hydrogels could be directed towards therapeutic roles because of their inherent anti-inflammatory properties.
**P161**

**Effect of Restoration on Electric Pulp Tester Stimulus in Pulpotomized Tooth**

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**Objectives** To evaluate the influence of coronal restoration after pulpotomy on the strength of stimulus from an electric pulp test (EPT) reaching to pulp space.

**Methods** The study used 10 orthodontically extracted mandibular premolar teeth from people aged 15 to 25 years. The teeth were cleaned and three millimetres of the apical root end were resected. Pulp tissue was removed by retrograde instrumentation and hypochlorite irrigation. A hole was drilled through the lingual root surface to reach the pulp canal space. The pulp space was dried and filled with electroconductive gel. The socket of typodont model was enlarged and extracted tooth was placed. The socket and base of typodont model were filled with alginate paste and the lip clip of an EPT was inserted. The cathode probe of Powerlab was inserted into the pulp space from the lingual opening and the anode was coupled to the EPT handpiece. The control dial of EPT was set at 5 and its probe wet with conducting media was placed at the occlusal third of the buccal tooth surface. The baseline readings of the electrical stimulus received at the pulp chamber were recorded in lab chart reader. The tooth was removed from the experimental model, access opening was performed and the pulp space was cleaned. A collagen sponge was placed inside the chamber up to the level of the cementoenamel junction. Pro Root MTA was mixed and 2 mm thick layer was placed. After the setting of the MTA the access cavity was restored with composite resin. Thereafter, the experimental setup was re-established. The pre and post pulpotomy electrical stimulus (in voltage) measurements at EPT reading 40 were recorded.

**Results** The data collected was analyzed using Stata software. Wilcoxon signed-rank test was performed to compare the mean pre and post pulpotomy electrical conductance of teeth. The electrical conductance (Mean, SD) of pre and post pulpotomized teeth were 0.0478 (0.03) V and 0.0486 (0.03) V. There was no statistically significant difference (P value= 0.95) between the pre and post pulpotomy readings.

**Conclusions** The placement of pulpotomy agent and composite restoration to seal access opening after pulpotomy have no effect on the strength of electrical stimulus reaching the pulp space during pulp sensibility test using EPT.

**P162**

**Human Dental Pulp Cells Release the Alarmin Adenosine Triphosphate (ATP).**

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**Objectives** Extracellular adenosine triphosphate (ATP) acts as an alarmin and may provoke painful sensations via interaction with purinergic receptors expressed on pulpal nerves. Tissue inflammation or injury has been shown to evoke ATP release from various cell types, but much remains to be learned about the stimuli that evoke ATP release from human dental pulp cells (DPCs). This study aimed to investigate extracellular ATP release from human DPCs following mechanical stimulation and/or treatment with lipoteichoic acid (LTA) or lipopolysaccharide (LPS).

**Methods** DPCs obtained from human extracted third molar teeth were cultured until they reached 70 – 90% confluency. DPC were treated with LPS [1 µg/mL] or LTA [10 µg/mL] and exposed to mechanical stimulation using an orbital shaker for 30 minutes. ATP release from DPC was measured immediately using an ATPlite luciferase assay.

**Results** ATP release from DPC was increased by a combination of LTA treatment and mechanical stimulation compared to untreated controls (*P<0.05). No increase in ATP release from DPCs was observed by LPS treatment. **Conclusions** This work provides evidence that ATP is released from human DPC following mechanical stimulation and treatment with the Gram-positive bacterial mimetic, LTA. Interestingly, antagonists for the purinergic receptor, P2X3, are in clinical trials for medical usage and have been shown to be well tolerated by patients. The relevance of these antagonists for dental pain is worthy of further investigation.
P195

Osteogenic Differentiation Potential of Diabetic Human Dental Pulp Stem Cells

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Objectives To investigate the influence of DM on DPSCs isolated from diabetic patients in comparison to non-diabetic controls

Methods The DPSCs were isolated from permanent teeth of non-diabetic and diabetic donors (n=3 for each) after informed patients’ consent and ethical approval from the Leeds Dental and Skeletal tissue bank. The two cell populations were comparatively evaluated for their proliferation rate (population doublings, PDT), and clonogenic ability (colony-forming unit fibroblast assay, CFU-Fs). Both cell groups were assessed for mesenchymal stem cells surface markers expression using flow cytometry. The osteogenic differentiation potential was evaluated after culturing the cells (at passage 4) in osteogenic inductive media at 2, 3 & 4 weeks incubation time points, using the Alizarin red stain quantification assay (ARS) & alkaline phosphates (ALP) stain.

Results The non-diabetic DPSCs had significantly higher CFU-F and lower PDT compared to the diabetic population group (p< 0.05). Both cell populations were expressed positive for CD73, CD90, and CD105 and negative for CD14, CD19, CD34, CD45 and HLA-DR, with no significant difference in the level of expression. After 14 days of osteogenic induction, both cell groups showed higher intensity ALP staining in gross appearance & microscopic images compared to the basal conditions. No significant difference was found between nondiabetic and diabetic cells after 3 and 4 weeks of incubation under osteogenic compared to basal conditions.

Conclusions DM had an effect on cell proliferation and colony-forming ability of dental pulp stem cells but not on their osteogenic potential. This indicates the need for further investigation into the impact of DM on the bone regenerative potential of these cells.

P163

Saliva Composition in Dental Caries and Oral Cancer Using (FT-IR) Spectroscopy

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Objectives To analyze stimulated saliva from patients with head and neck cancer (HNC), dental caries (DC), and in healthy individuals (H) with (ATR) FT-IR spectroscopy.

Methods Four different groups were included: 14 individuals diagnosed with HNC, 10 individuals with DC, 10 H individuals 20-40 years old and 10 H individuals 40-66 years old. Stimulated saliva was collected for 5 min, whereafter it was centrifuged and the pH measured. Total protein concentration was analyzed using a BCA protein kit. The saliva samples were lyophilized prior to FT-IR analysis performed.

Results The highest mean secretion rate was seen for the healthy group 20-40 years old, 3.5±1.6 ml/min. For the DC, the mean secretion rate was lowest 1.6 ± 1.0 ml/min while it was 2.1 ± 0.2 ml/min in the HNC group. Comparable mean total protein concentrations were found in the healthy groups, 0.9 ±0.2 mg/ml (20–40 years old) and 1.0 ± 0.1 mg/ml (40-66 years old). The mean protein concentration was higher in HNC 2.4 ± 0.1 mg/ml. A slightly lower mean pH was seen for the HNC compared with the other groups (pH 7.0 ± 0.4). FTIR (ATR) showed that all groups had as expected the protein bands Amide I, Amide II and Amide III (1650–1450 cm⁻¹). However, the intensity varied greatly depending on the sample weight and group. Lipids from bacterial cells were detected in both the caries and HNC groups, but not in the healthy groups. Furthermore, ester- and thiocyanate function groups were more distinct in patients with either dental caries or HNC compared with the healthy groups.

Conclusions The largest differences between the groups were seen for lipids and ester functional groups compared to healthy individuals. This indicates that in non-healthy conditions in the oral cavity altered proteins and fat-soluble fragment might exist
Saliva Improves in Vitro Protein Digestibility of Buriti-Containing Yoghurt

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Objectives Buriti (Mauritia flexuosa) is an under-utilized Amazonian fruit with a high content in phenolic compounds. Although polyphenols have health benefits, they may also hinder protein digestibility but salivary proteins might counteract this negative effect through binding to polyphenols. The objective of this work was to describe the impact of saliva on protein digestibility of yoghurt containing buriti.

Methods A water extract of buriti flour was mixed with human saliva (LeeBiosolutions) at estimated concentrations varying from 0.001 to 0.05 g polyphenols (GAE)/ml. The particle size distribution in the mixtures was measured by laser diffraction. Pepsin and trypsin activity were measured in presence or not of the buriti extract. Furthermore, an in vitro model of digestion was applied to yoghurt or to yoghurt with buriti flour (1:4 w/w). In the oral phase of digestion, the two food products were mixed with human saliva or simulated salivary fluid SSF (ionic solution) at a ratio of 1:1. The standardized INFOGEST static model was then performed. The gastric and intestinal phases of digestion were conducted using pepsin and pancreatin, respectively. Digestive proteolysis was assessed by measuring accessible NH₂-groups in samples over time by the OPA method.

Results Buriti extract had no significant effect on pepsin or trypsin activity. However, when added to yoghurt, buriti flour had a large inhibiting effect on digestive intestinal proteolysis. Interestingly, the presence of saliva instead of SSF during digestion of buriti-containing yoghurt increased slightly the digestive proteolysis, both in the gastric and intestinal phase. Finally, buriti extract had no detectable impact on particle size in saliva (D₅₀ ~ 45µm), suggesting no major aggregation of salivary proteins.

Conclusions Compared to SSF, saliva improved protein digestibility of yoghurt containing buriti flour, but is unclear whether this occurred through binding of salivary proteins to polyphenols. Further investigations are required.

Evaluation of Mandibular Bone Density in Bruxers on Panoramic Radiographs

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Objectives This cross-sectional study aimed to establish a difference in mandibular bone density between bruxer and non-bruxer patients, based on panoramic radiographs.

Methods Panoramic radiographs of bruxer and non-bruxer adult patients were analyzed with the medical imaging analysis software ImageJ®. A specific region of interest located directly below the first mandibular premolar was defined. The radiographs were evaluated by two blinded, calibrated evaluators (Bland-Altman test and Cohen’s Kappa for checking the inter-evaluators reproducibility). Several radiological determinants were studied on the patients’ panoramic radiographs: gray values of cancellous bone and cortical bone at the level of the region of interest (Welch’s t-test), and the presence or absence of bony exostoses at the mandibular angle (Chi-square test).

The authorization of the Data Protection Officer of the AP-HM was obtained for the collection and processing of data, which were anonymized (registered in the RGPD-Ap-hm registry under number 2020-149).

Results Eighty-four panoramic radiographs representing the 84 patients were evaluated for this study (37 bruxers and 47 non-bruxers). There was a good inter-evaluator reproducibility. A statistically significant difference (p < 0.05) was noted in the cancellous to cortical bone ratios of bruxers and non-bruxers: the density of cancellous bone was greater in bruxers than in non-bruxers. The number of bony exostoses at the mandibular angle was significantly higher in bruxers (p < 0.05).

Conclusions Based on the examination of panoramic radiographs, this study has shown an association between bruxism and bone development in the mandible, with a greater cancellous bone density and the presence of bony exostoses at the mandibular angle in the bruxer patient. Further studies are needed to supplement this preliminary approach, especially via the analysis of three-dimensional imaging, such as Cone Beam Computed Tomography, to overcome the limitations of panoramic radiography.
Association Between EBV Infection and Risk Factors in OSCC Patients
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Objectives Oncogenic viruses such as Human papillomavirus and Epstein-Barr virus (EBV) have been linked to a subset of oral squamous cell carcinoma (OSCC), especially in patients who have no traditional risk factors for OSCC development. This study aimed to investigate the EBV infection in OSCC cases, as well as the association between risk-factor status and the presence of EBV, in Thai patients with OSCC.

Methods Twelve formalin-fixed paraffin-embedded (FFPE) specimens of OSCC from patients with no traditional risk factors (e.g., smoking, alcohol consumption, and betel nut chewing), as well as twelve FFPE specimens of OSCC from sex-matched patients with risk factors, were retrospectively obtained. DNA extraction was performed; this was followed by polymerase chain reaction analysis to determine EBV DNA status, using primers specific for the LMP-1 gene. Clinicopathological information for all cases, including patient age, sex, tumor location, and histological grading, was collected. Statistical analysis was performed using McNemar’s test. p-values < 0.05 were considered statistically significant.

Results Of the 24 OSCC specimens, six (25%) exhibited EBV DNA. Regarding the risk-factor status of patients with OSCC, four of twelve (33.33%) OSCC cases with no traditional risk factors and two of twelve (16.67%) OSCC cases with risk factors exhibited EBV DNA. However, no significant association between risk-factor status and EBV presence could be established in this group of OSCC patients.

Conclusions The prevalence of EBV presence was 25% in this group of OSCC patients; no association between risk-factor status and EBV presence was identified. Further studies with a larger sample size are required to clarify this notion.
Salivary Mucoepidermoid Carcinoma: a Clinicopathological Study of 38 Cases
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Objectives Salivary gland tumors (SGTs) are a group of lesions with heterogenous histological features and biological behaviors that comprises about 5% of neoplasms in head and neck region. Mucoepidermoid carcinoma is the most common malignant SGT in both adults and children. This study aimed to describe the diversity of clinicopathological features and survival outcomes of salivary MEC to better characterize this neoplasm.

Methods This study was approved by the local research ethics committee (CAAE: 74754317.5.0000.5335). A retrospective analysis of all salivary MEC was performed from the histopathological archives of the Santa Casa de Misericórdia de Porto Alegre, Rio Grande do Sul, Brazil. Clinicopathological features were retrieved, and histological slides stained with hematoxylin and eosin reviewed to confirm the diagnosis. All tumors were histologically graded according to Goode et al. (1989). The Kaplan-Meier method was used to estimate survival rates, and the difference between survival curves was investigated by the Log-Rank test.

Results A total of 38 MEC were included, being 24, 7, and 7 cases graded as low-, intermediate and high-grade, respectively. A female predilection (n=26) was observed, with an average age of 46.21 years. The parotid gland was the involved site in the majority of cases (n=30) and an asymptomatic nodule (n=29) was the most common clinical appearance reported. Perineural and vascular invasion were found in one and eight cases, respectively. Most tumors were diagnosed in stage I or II (n=27) and surgery was the main treatment employed (n=16). At the last follow-up, 26 patients were free of disease and four presented relapses of disease. The 5-year disease-free survival was 90%.

Conclusions MECs usually have a low-grade histological presentation, most commonly affecting the parotid glands in middle-aged women. The recognition of the clinicopathological features of MEC is helpful for an accurate diagnosis of this carcinoma.
Delayed Post-Extraction Healing in Mice Lacking Initial Oral Mucosal Lymphatics
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Objectives Lymphatics are important for resolution of inflammation, but their role in the oral wound healing process is insufficiently studied. The aim of this study was to investigate mucosal and bone healing responses following extraction of maxillary second molars in a transgenic mouse model lacking initial lymphatics in oral mucosa.

Methods Second bilateral maxillary molars were extracted in wild type (WT, C57/Bl6) and transgenic K14-VEGFR3-IgG mice. The animals were sacrificed on day 7, 14 or 21 post-extraction and jaws were removed. Mucosal wound closure was clinically assessed and measured under a photomicroscope. Bone healing was evaluated by micro-CT analysis following PFA fixation. Inflammatory infiltrates were immunohistochemically evaluated in the same jaws. Proteins were extracted from contralateral jaws and subjected to proteomic profiling and multiplex analyses for different cytokines.

Results Wound closures occurred faster in WT than transgenic mice, with a clear difference on day 14. Trabecular bone developed in both mice strains over 21 days, it was however significantly decreased after 14 days in K14-VEGFR3 mice compared with WT controls. Bone mineral density was also significantly lower at day 14 in the transgenic group. Rich infiltrates of neutrophils were seen at day 7 in both groups, with increased infiltrates in transgenic mice up to 14 days in wounds with incomplete healing. Blood vessels were abundant at all observation times in both mice strains, while lymphatics were identified within the wounds of WT mice alone. Epithelial growth factor (EGF) expression was significantly higher in WT compared with transgenic mice at all observation times, while C-reactive protein (CRP) showed elevated values in K14-VEGFR3 compared with WT mice. Chemokine-ligand 21 (CCL21) was only expressed in WT gingiva, and significantly higher in WT bone samples compared with transgenic samples.

Conclusions Lack of initial lymphatic vessels delayed wound healing responses in mucosa and bone after tooth extractions in mice.
Salivary Opiorphin Levels After Capsaicin Stimulation in Orofacial Pain Patients and Controls
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Objectives Opiorphin is an endogenous inhibitor of opioid degrading enzymes released by salivary glands and displaying strong analgesic properties. This study is aimed at comparing the intensity of burning pain sensation and salivary opiorphin levels after oral stimulation with capsaicin in burning mouth syndrome (BMS), chronic temporomandibular disorders (TMD) patients and healthy subjects.

Methods Forty subjects participated (8 BMS, 16 TMD-pain patients and 16 controls). Burning pain sensation was elicited by putting a series of 10 capsaicin-soaked disks (30µM) in contact with the dorsal tongue, and the intensity was recorded with a Numerical Pain Rating Scale (NPRS) every minute during a 5 minute application period and every 2 minutes during next 20 minutes upon termination of capsaicin stimulation. Saliva sampling for opiorphin levels was performed on three time points, before and twice following the stimulation. Opiorphin levels were quantified by HPLC-MS/MS. Analysis of variance (ANOVA), Repeated measurements ANOVA and post hoc-tests were used for data analysis.

Results The values of experimental burning pain sensation (peak values at 5th measurement): controls =-4.56±0.42; TMD=4.13±0.56; BMS=6.13±0.95) differed significantly between groups (p=0.0049), with significantly higher values in BMS group (BMS vs.TMD p=0.0052; BMS vs. controls p=0.0165). BMS patients showed statistically higher levels of opiorphin, compared to controls in second (BMS:3.47±1.18pg/ug; control:0.90±0.19pg/ug; p=0.035) and third sampling (BMS:4.58±1.84pg/ug; control:0.90±0.15pg/ug; p=0.014). Also, in BMS group, a statistically significant increase in opiorphin levels was observed between the 1st and 3rd sampling (p=0.0166).

Conclusions BMS patients experienced a more intense pain sensation after tongue stimulation by capsaicin than TMD patients and healthy control subjects. Opiorphin levels were higher in orofacial pain patients than in healthy subjects, and BMS patients displayed higher levels than TMD patients. These results indicate a specific regulation of opiorphin release in patients with oral painful conditions.

COVID-19 Infection Does not Worsen Symptoms of Temporomandibular Disorders
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Objectives In order to assess the potential relationship between COVID-19 and temporomandibular disorders (TMD), we aimed to investigate whether TMD symptoms and oral behaviors (OB) may have increased in TMD patients during the two-year period of the COVID-19 pandemic. This relationship may be a consequence of the infection itself, or, since stress is a risk factor for both TMD and OB, due to psychological stress that a pandemic period might cause in general.

Methods An online survey was sent to our cohort of 186 previously diagnosed TMD patients (DC/TMD). The questions in the survey addressed the possible TMD symptoms and OB changes during the past 2 years of the COVID-19 pandemic, and patients’ tendency to feel depressive or anxious during the pandemic. We compared the obtained data between patients who had COVID-19 infection with those who didn’t. Chi-square test and Spearman’s correlation were used for data analysis.

Results The survey response rate was 63.98%; 57.9% of individuals confirmed having had a COVID-19 diagnosis and 42.1% denied COVID-19 infection. The percentage of subjects who developed new TMD symptoms or their symptoms worsened during the pandemic was 40.33%. The percentage of individuals reporting an increase in OB was 27.73% for day-time grinding, 26.89% for night-time grinding, and 19.33% for other habits. There weren’t significant differences in the presence of participants with reported new symptoms (TMD symptoms or OB) between those that previously had COVID-19 diagnosis and those that weren’t infected (p>0.05). Also, no correlation was present between symptoms of anxiety/depression and previous COVID-19 diagnosis (p>0.05).

Conclusions TMD and OB showed an increase during the period of the COVID-19 pandemic, but previous COVID-19 diagnosis isn’t associated with the development of new or worsening of existing TMD symptoms or OB. The cause of the worsening of TMD symptoms and OB during the pandemic is likely a consequence of stress caused by the pandemic in general.
Gene Polymorphisms Associated With Increased Hypervigilance in Temporomandibular Disorders Patients

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¹University of Zagreb, School of Dental Medicine, Zagreb, Croatia, ²Department of Prosthodontics, School of Dental Medicine, University of Zagreb, Zagreb, Croatia, ³Department of Removable Prosthodontics, School of Dental Medicine, University of Zagreb, Zagreb, Croatia, ⁴Division of Molecular Medicine, Rudjer Boskovic Institute, Zagreb, Croatia

Objectives Temporomandibular disorders (TMD) are chronic conditions of unknown etiology. Genetic factors have been increasingly proposed as risk factors in the etiopathogenesis of TMDs, which are often associated with certain psychological characteristics. One of them is hypervigilance, a state of powerful and unpleasant increased alertness, manifested as a state of high amplification of typical somatic experience. The aim was to investigate association of certain genetic polymorphisms (SNPs) with hypervigilance and sensitivity in TMD patients.

Methods Study included 108 subjects (59 TMDs and 49 controls). After establishment of the initial diagnosis of TMD according to DC/TMD, swabs of buccal mucosa were taken from all subjects. Genomic DNA was extracted by a previously established method. Polymorphisms in two genes, opiorphin (OPRPN, rs1387964) and fatty acid amide hydrolase (FAAH, rs932816) were genotyped by the TaqMan SNP Genotyping method. The Brief Hypervigilance Scale (BHS) and Somatosensory Amplification Scale (SSAS) were completed by all subjects. Kruskal–Wallis test and Mann–Whitney U test were used for data analysis.

Results Genotypes rs1387964: a) total: TT 61%, CC 6.5%, TC 32.5%; b) TMD: TT 65.5%, CC 7%, TC 27.5%; c) healthy subjects: TT 55.1%, CC 6.1%, TC 38.8%. BHS and SSAS scores were generally lowest in the CC group and highest in the TC group. In the TMD group, heterozygotes with the TC genotype had statistically significantly higher hypervigilance when compared to homozygous patients (TT=3.21, CC=2.5, TC=6.75; Kruskal–Wallis H(2)=9.43, p=0.0089).

Genotypes rs932816: a) total: GG 57.4%, AA 7.4%, GA 35.2%; b) TMD: GG 61.2%, AA 8.3%, GA 30.5%; c) healthy subjects: GG 50%, AA 5.6%, GA 44.4%. Homozygotes carrying two A-alleles generally presented higher BHS and SSAS scores, when compared to patients carrying the other two genotypes. TMD patients, AA homozygotes, presented significantly higher BHS scores when compared to patients carrying the two other genotypes (GG=5.09, AA=8, GA=1.9; Kruskal–Wallis H(2)=7.63, p=0.022).

Conclusions Specific polymorphisms in the OPRPN and FAAH genes are significantly associated with the score of the hypervigilant state, in TMD patients. Our results indicate a strong connection between specific genetic background and variables related to psychological aspects of the chronic TMD.
Expression of DAPK1 in Oral Leukoplakia and Oral Cancer

ATHANASIOS POULOPOULOS1, Petros Papadopoulos1, Apostolos Chatzivasileiou1, Dimitrios Parlitsis1, Elefterios Anagnostou1, Pinelopi Anastasiadou1, Konstantinos Vaxtsevanos2, Marie Bolon1

1ORAL MEDICINE & ORAL PATHOLOGY, ARISTOTLE UNIVERSITY OF THESSALONIKI, Thessaloniki, Greece, 2Oral & Maxillofacial Surgery, ARISTOTLE UNIVERSITY, SCHOOL OF DENTISTRY, Thessaloniki, Greece

Objectives Death-Associated Protein Kinase 1 (DAPK1), a serine/threonine (Ser/Thr) kinase participates in a wide range of cellular processes including apoptosis and tumor suppression. Limited information is available regarding oral precancerous and oral cancer lesions.

Methods DAPK1 antibody (dilution 1/50, NBP2-38468, NovusBiologicals LLC, Centennial, CO, USA) was used for this immunohistochemical study. The study included paraffin embedded samples of 18 OSCC and 32 OL cases compare to 20 histologically oral normal mucosa samples. The samples had been provided by the archives of the Dept of Oral Medicine / Pathology, Dental School, Aristotle University of Thessaloniki, Greece. The results described the expression pattern of DAPK1 through SPSS Mann-Whitney U Test statistical analysis (p<0.05).

Results The expression of DAPK1 was similar in both normal epithelium and in epithelium of OL with mild or without dysplasia (p=0.336). In contrast, it was significantly higher in normal epithelium compared to OL with moderate and severe dysplasia (p=0.019), respectively. Moreover, DAPK1 expression was much higher in the epithelium of OL with no dysplasia compared to OL with moderate to severe degree of dysplasia (p=0.043). The expression of DAPK1 in OSCCs was observed with positive neoplastic cells in the center of neoplastic epithelial islands and especially around keratin pearls. Overall, OSCCs DAPK1 expression showed a statistically significant decrease in OSCCs compared to normal epithelium (p=0.04) and OL without (p=0.002)/mild dysplasia (p=0.02) but not compared to OL with moderate (p=0.921)/severe (p=0.999) dysplasia.

Conclusions Lower expression of DAPK1 in OSSCs in comparison with potentially malignant lesions (Leukoplakia) or normal epithelium confirms its tumor suppressive activity. A different pattern of expression was found between OL with no and mild dysplasia and OL with moderate severe dysplasia indicate a possible early diminished role of DAPK1 suppression leading to more severe dysplasia and malignancy of oral epithelium. This characteristic finding of DAPK immunohistochemical expression in OL could be used as an indicator marker of their potential malignant transformation.
Osteomas of the Jaws: Predictive Sign of Gardner’s Syndrome?

Silvia D’agostino1, 2, Luisa Limongelli1, Gianfranco Favia2, Marco Dolci1

1Medical, Oral and Biotechnological Sciences, University of Chieti Pescara, Chieti, Italy, 2Department of Interdisciplinary Medicine, University A. Moro, Bari, Italy

Objectives Gardner Syndrome (GS) is a combination of polyposis, osteomas, fibromas and sebaceous cysts. The aim of the study is to highlight if maxillofacial osteomas could represent an early detection symptom of GS.

Methods Inclusion criteria: single or multiple osteomas hypothesis in the upper/lower jaw to be confirmed by histological analysis, osteomas of the jaws histologically diagnosed, positivity to APC gene mutation test. After the WHO eight-step intraoral examination, the following clinical data were collected: age, gender, intra-and extraoral osteomas locations and numbers, cranio-facial pain, facial asymmetry, intestinal polyposis (FAP), colorectal cancer (CRC) and family history of FAP/CRC, soft tissue lesions, referred symptoms were also collected. Panoramic radiographs were performed in all instances associated with CBCT if necessary. Bone samples were collected from osteomas.

Results The database gathered 19 patients with oral osteomas. All details about patients are extensive reported in Table 1. Every patent was positive to APC gene mutation analysis. Mean age of 47 (±17.08) years, 52.6% (10) men and 47.3% (9) women. A total of 24 oral osteomas was found, of which 66.7% (16) in the mandible and 33.3% (8) in the maxilla. 26.3% (5) showed other cranial locations, with a total of 6 cranial lesions. While 36.8% (7) reported peripheral osteomas such as clavicle, humerus, rib, ulna, tibia and fibula. No patient reported cranio-facial pain and 31.6% (6 patients) revealed facial asymmetry. 68.4% (13) had polyposis, while only 5.2% (1) developed CRC. 21.1% (4) denoted dermoid cysts, whereas only 10.5% (2) displayed epidermoid cysts.

Conclusions The whole sample presented osteomas of the jaws and/or the facial skeleton. These results agree with the hypothesis that osteomas of the jaws are a predictive sign of GS.
<table>
<thead>
<tr>
<th>Patient #</th>
<th>Sex</th>
<th>Age</th>
<th>Characteristics</th>
<th>Oral location</th>
<th>Other cranial sites</th>
<th>Other sites</th>
<th>Cranio-cervical pain</th>
<th>Facial asymmetry</th>
<th>Intracranial hypoplasia</th>
<th>CCA</th>
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**Declaration Of Helsinki and Ethical Committee Approval**

1. **Mandible osteoma diagnosis** to be confirmed by histological analysis.
2. Osteomas diagnosed from 2016 to 2021.
3. **ABC gene test**

**Age, gender, osteoma locations and maxillary sinusal, facial asymmetry, intraoral polyps, Ca and lung metastasis, referred symptoms**

**OPT/CBCT**

**Osteoma biopsy**

**Customized follow-up**
Smile Evaluation on Videographs: an Intra and Inter-Rater Agreement Study
Mathias FAURE-BRAC, Angéline Antezack, Sebastien Melloul, Mehdi Saïd, Anne Raskin, Virginie Monnet-Corti
Periodontology, Aix-Marseille University, Marseille, France
Objectives The aim of this study is to reproducibly evaluate on videographs an aesthetic score (Smile Esthetic Index (SEI)) previously validated on photos.
Methods Smile videographs were done using a smartphone associated with the Smile lite MDP mounted on a tripod. They were then randomized and evaluated twice consecutively at a 1-week interval by 3 periodontists according to the SEI based on 10 variables. Cohen's Kappa and Fleiss' Kappa tests were performed to measure intra- and inter-rater agreement.
Results Sixty-five smile videographs of 24 men and 41 women (mean age 33 +/- 11.3 years) were scored. A mean intra-individual agreement of 0.68 [0.64-0.73] was obtained, representing substantial agreement. The inter-individual agreement calculated for each variable ranged from 0.31 for the variable "absence of visible excessive gingiva" to 0.90 for the variable "absence of diastema and/or missing inter-dental papilla."
Conclusions We have shown that it was possible to evaluate reproducibly on videographs an aesthetic score (SEI) previously validated on photos.

Intra-Oral Scan Plaque Score Study Comparing Operators and Indices
Natasha West, Joon Seong, Nicola Hellin, Robert Newcombe, Charles Parkinson, Nicola West
1University of Bristol, Bristol, United Kingdom, 2Cardiff University, Cardiff, United Kingdom, 3GSK, Weybridge, United Kingdom
Objectives To compare clinical 2 & 4-point O'Leary plaque scores with simultaneous intra-oral scan (IOS) (TRIOS®) plaque images scored later, then re-scored.
Methods A gold standard clinician (1) assessed 30 participants' O'Leary plaque scores 6-6 both arches. Clinician 1 and a trained, calibrated inexperienced clinician (2) scored plaque from their IOS images (session 1), repeating two weeks later (session 2).
Results Clinician 1 achieved excellent agreement between clinical and IOS plaque scores, with just 2 (session 1) & 0 (session 2) of 1294 sites differing. Corresponding analyses for clinician 2 show very substantial agreement with clinical scores, with 21 & 18 disagreements. 2-point scores reconstructed from 4-point scoring agreed closely with original 2-point scores, with 1 (clinical), 7 & 3 (clinician 1 IOS) and 31 & 18 (clinician 2 IOS) disagreements. Mean clinical 2-point scores for Ramfjord teeth and all teeth were highly correlated (r +0.8) with means differing by 0.01.
Conclusions Inexperienced clinicians can be trained rapidly to obtain comparable, clinically relevant accuracy. We confirm the potential versatility of recruiting clinicians/recorders to score IOS plaque images remotely for clinical studies or practice, potentially reducing chairside time for patient and operating clinician. Potentially 4-point scoring could discriminate better between anti-plaque treatments, by accommodating a greater gradation of response; conversely our findings suggest possible ambiguity of demarcation between mesial and distal areas. Scoring Ramfjord teeth alone would give similar results, in line with parallel findings based on discrimination between effects of interventions.
Clinical Gingival Health Improvement From Behavior Modification and Anti-Gingivitis Toothpaste

Joon Seong1, Sinéad Daly1, Nicola Hellin1, Robert Newcombe1, Charles Parkinson2, Nicola West1

1University of Bristol, Bristol, United Kingdom, 2GSK, Weybridge, United Kingdom, 3Cardiff University, Cardiff, United Kingdom

Objectives This study evaluated oral hygiene behaviour modification in combination with an anti-gingivitis toothpaste on gingival health compared to the standard of care provided in general dental practice with utilisation of preferred home care products (control group) over 6 months.

Methods A gold standard clinician (blind) assessed >80 adult participants in a parallel, randomised controlled trial with early gingivitis, recording bleeding on probing (2 sites) and plaque scores (Silness & Löe 1964) at 4 sites/tooth for 6-6 in both arches at 4 visits over 6 months (baseline, 3 weeks, 3 and 6 months). Participants were scored before and after toothbrushing at each visit. The test group received oral hygiene instruction (OHI) with the aid of an intra-oral scan of their mouth and brushed with sodium bicarbonate anti-gingivitis toothpaste compared to the control group who receive oral hygiene instruction according to the standard of care in general dental practice, brushing their teeth with their preferred home use toothpaste.

Results 85 participants were enrolled and randomised, producing the expected degree of balance on demographic characteristics, 83 providing follow-up data, with no adverse events. The intervention resulted in significant improvement in bleeding and plaque scores relative to the control group (p<0.001) over 6 months, with both test and control groups improving over time for both indices.

Conclusions This study demonstrates the benefit of oral hygiene behaviour modification combined with the use of a sodium bicarbonate based anti-gingivitis toothpaste to improve overall gingival health, compared to the standard of care provided in general dental practice with utilisation of preferred home care products.

Endotoxin Activity in Subgingival Plaque Matches Periodontal Diagnoses

Anbo Dong1, Pirkko Pussinen2, Susanna Paju2, Jaakko Leskelä2, Gordon Proctor1, Svetislav Zaric1

1King’s College London, London, United Kingdom, 2University of Helsinki, Helsinki, Finland

Objectives To compare the endotoxin activity in subgingival plaque samples in individuals with healthy periodontium, gingivitis patients and patients with periodontitis and correlate this with the severity of the disease.

Methods Subgingival plaque samples from 324 individuals recruited for the cross-sectional SECRETO Oral study were assessed for their endotoxin activity, using the recombinant Factor C assay (ENDOZYME II GO, bioMérieux). The participants were 18-49 years of age and all undergone a full periodontal assessment with plaque collection from the deepest periodontal sites. For diagnostic purposes, the 2017 periodontal disease classification was used. Statistical differences of endotoxin activities between different diagnostic groups and stages of periodontitis were assessed using the one-way analysis of variance (ANOVA).

Results Endotoxin activity in subgingival plaque samples from individuals with healthy periodontal tissues was significantly lower compared to patients with gingivitis and periodontitis (1138 EU/ml, 2605 EU/ml, and 2986 EU/ml respectively). In addition, subgingival endotoxin activity correlated well with the stage of periodontal disease, with endotoxin activity of stage 1 patients significantly lower than of those with more severe disease (S1-1546, S2-2786, S3-3105, and S4-3825 EU/ml). The prognostic value of this biomarker is yet to be examined.
Efficacy of Different Periodontal Prognostic Systems in Predicting Tooth Loss

Selai Saydzai, Luigi Nibali, Zoe Buontempo, Pankti Patel, Hasan Fatemah, Sun Chuanming, Aliye Akcali, Nikolaos Donos, Guo-Hao Lin

1Faculty of Dentistry, Oral & Craniofacial Sciences, King’s College London, London, United Kingdom
2Department of Periodontology, Faculty of Dentistry, Suzhou Health College, Suzhou, China
3Department of Periodontology, Dokuz Eylül University, Faculty of Dentistry, Izmir, Turkey
4Centre for Oral Clinical Research, Barts and The London School of Medicine & Dentistry, Queen Mary University of London (QMUL), London, UK
5Department of Orofacial Sciences, School of Dentistry, University of California San Francisco, California San Francisco, California, United States

Objectives

The aim of this analysis was to assess how different tooth prognosis systems could predict tooth loss in a cohort of periodontitis patients followed prospectively during supportive periodontal care (SPC).

Scientific rationale for study: Evidence is limited on reproducibility and predictive ability of simple periodontal tooth prognostic systems for chair-side use.

Methods

Clinical and radiographic data of 97 patients undergoing regular SPC for 5 years were used to assign tooth prognosis using four different systems (McGuire & Nunn 1996, Kwok & Caton 2007, Graetz et al. 2011, Nibali et al. 2017). Three independent examiners assigned tooth prognosis using all four systems, following a calibration exercise. Their association with tooth loss was tested.

Results

The Intraclass Correlation Coefficient for all 4 systems and 3 examiners was very high, showing good reproducibility. Based on available data that has been collected throughout the duration of the research, all four systems showed a strong ability to predict tooth loss during 5 years of SPC. The 97 patients included in this report had 2318 teeth at baseline (excluding third molars), 31 of which (1.37%) were extracted throughout the study.

Principal findings: The studied prognostic systems showed good reproducibility and ability to identify teeth at high risk of tooth loss during 5 years of supportive periodontal care. However, sensitivity was low, as many teeth identified as hopeless were retained.

Conclusions

This study shows that the investigated prognostic systems can be reliably used to identify teeth at higher risk of being lost in patients undergoing SPC, also when used by inexperienced dental students. Prognostic systems may help to manage patient expectations, save time for both clinician and patient and limit the financial implications of complex periodontal disease.

Practical implications: The use of these prognostic systems, with some suggested modifications, is advisable as a means to establish tooth prognosis.

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for outcome tooth loss at 5 years. ‘Hopeless’ (or ‘unfavourable’) categories were compared to all other categories.

<table>
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<tr>
<td><strong>Sensitivity</strong></td>
<td>3.23% (0.08% - 16.70%)</td>
<td>3.23% (0.08% - 16.70%)</td>
<td>12.90% (3.63% - 29.83%)</td>
<td>9.68% (2.04% - 25.75%)</td>
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<tr>
<td><strong>PPV</strong></td>
<td>25.00% (3.44% - 75.71%)</td>
<td>33.33% (4.45% - 84.30%)</td>
<td>18.18% (7.39% - 38.22%)</td>
<td>13.64% (4.69% - 33.61%)</td>
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<td><strong>NPV</strong></td>
<td>98.67% (98.58% - 98.75%)</td>
<td>98.67% (98.58% - 98.75%)</td>
<td>98.79% (98.62% - 98.94%)</td>
<td>98.74% (98.59% - 98.88%)</td>
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</table>

Definitions: Sensitivity - ability to identify a hopeless tooth (if assigned a hopeless/unfavourable prognosis, it will be lost) Specificity - ability to identify a non-hopeless tooth (if assigned a non-hopeless/unfavourable prognosis, it will not be lost) PPV - If a tooth is in the hopeless/unfavourable categories, how likely the tooth is lost NPV - If a tooth is in the non-hopeless/unfavourable category, how likely the tooth is not lost
Evaluation of Pain After Periodontal Surgery
Stefano Gennai, Marco Nisi, Rossana Izzetti, Filippo Graziani
University of Pisa, Pisa, Italy

Objectives To evaluate levels of pain and associated discomfort during and following various types of surgical periodontal procedures.

Methods The sample consisted of 103 patients submitted to different surgical periodontal treatments (i.e. conservative, regenerative, muco-gingival and resective). The patients registered pain using a Visual Analogue Scale (VAS) and a Numerical Rating Scale (NRS) immediately after the conclusion of the procedure. A written questionnaire about Oral Health Impact Profile (OHIP-14), post-operative pain score and discomfort score was compiled 24h after treatment. The patients also recorded if analgesics were taken.

Results Mean NRS score resulted 0.97 ±1.03 immediately after surgery. Data analysis showed a mean discomfort score of 7.34 ± 5.45, a mean post-op pain score of 5.46 ± 3.49 and an OHIP-14 score of 25.73 ± 9.55. Within 24h following the operation, patients reported as average value for oral pain 1.27 ± 1.23, and 1.33 ± 1.55 was found to be mean for the worst level of pain experienced.

Conclusions Levels of pain and discomfort were found to be low to very low for most patients after all the different types of surgical periodontal treatment; this also agreed with the low consumption of analgesics. Furthermore, the fact that the patients who received muco-gingival surgery were those for whom it took the longest could explain why they complained greater post-operative pain and discomfort. Lastly, resective treatments seemed to be associated with a worse Oral Health Impact Profile and a higher residual pain at 24h from the intervention, although a lack of statistical significance.

Is air-Polishing Clinically Relevant in Periodontal Active and/or Supportive Treatments?
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Objectives Active and supportive periodontal treatments conventionally use ultrasonic and mechanical devices whose effectiveness is well established. Air Polishing Devices (APDs) are indicated for plaque removal, polishing tooth surfaces and elimination of extrinsic stains. Low abrasive powders (erythritol, glycine, trehalose) allow subgingival instrumentation but evidence is scarce regarding their relevance in periodontal treatment. The aim of this systematic review was to assess the clinical relevance of APDs during active and/or supportive treatments in addition or in comparison to conventional instrumentation.

Methods Following PRISMA guidelines, a literature search was performed in April 2022 using the PubMed/MEDLINE databases on articles published in the last ten years. The inclusion criteria were randomized controlled trials, systematic reviews or meta-analysis dealing with: air-polishing during periodontal active and supportive treatments, comparing air-polishing and ultrasonic instrumentation and assessing clinical outcomes (CAL, BoP, PPD).

Results Among the 20 articles included, 14 were randomized controlled trials (7 on active periodontal treatment and 7 on supportive periodontal treatment) and 6 were systematic reviews or meta-analyses (2 on active periodontal treatment and 4 on supportive periodontal treatment). For active periodontal treatment, the adjunctive use of air-polishing did not show any statistically significant difference when compared to ultrasonic debridement alone except in 1 study showing better results on PPD. For supportive treatment, the use of air-polishing did not show any statistically significant difference compared to ultrasonic debridement alone except for 1 study showing better results on PPD and CAL, but better scores were obtained for ADPs about patient pain and discomfort during and after treatment, using Visual Analogue Scale.

Conclusions In conclusion, the use of ADPs seems more relevant in supportive periodontal treatment because not inferior to conventional instrumentation, and better acceptance for patients and less time consuming were noticed.
Frondoside a Promotes Periodontal Healing After Delayed Tooth Replantation

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Objectives Tooth avulsion can cause severe damages to periodontal ligament and surrounding tissues. Such damages can lead to inflammatory resorption after tooth replantation. Frondoside A (FA) is a triterpenoid saponin isolated from edible sea cucumber, \textit{Cucumaria frondosa}. The purpose of this study was to elucidate the effect of FA on periodontal healing after delayed tooth replantation.

Methods Human periodontal ligament (PDL) cells were treated with 0.01, 0.03, 0.05, 0.1, 0.3 $\mu$M of FA for MTT assay. Next, maxillary molars from Sprague-Dawley rats were extracted and stored in milk for 60 mins. Afterwards, molars received endodontic treatment to minimize inflammatory root resorption associated with pulp necrosis. The molars were randomly divided into control group (no treatment) and FA group (topical delivery of 0.1 $\mu$M of FA immersed in absorbable gelatin sponges). The animals were sacrificed on either 7 or 28 days after the experiment. Both RT-PCR analysis for pro-inflammatory biomarkers (TNF-\(\alpha\), RANKL, NF-\(\kappa\)B p50, NF-\(\kappa\)B p65) and histological analysis were performed.

Results MTT assay confirmed that PDL cell viability was significantly decreased at a concentration of 0.3 $\mu$M FA for 48 hrs compared to the other concentrations ($p < 0.05$). Regarding pro-inflammatory biomarkers, FA group showed lower level of TNF-\(\alpha\) expression compared to the control ($p < 0.05$). In histological analysis, FA group showed significantly lower inflammatory root resorption rate compared to the control ($p < 0.05$).

Conclusions Topical delivery of FA promoted the periodontal healing of replanted teeth by inhibiting the expression of pro-inflammatory biomarkers and reducing the rate of inflammatory root resorption. Within the limits of this study, topical delivery of FA can be beneficial to delayed tooth replantation.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{CellViabilityTest.png}
\caption{Cell viability test. MTT assay confirmed that concentrations below 3 $\mu$M of FA maintained PDL cell viability. * $p < 0.05$}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{ExpressionOfBiomarkers.png}
\caption{Expression of pro-inflammatory biomarkers by RT-PCR. Among the biomarkers, FA group showed significantly lower level of TNF-\(\alpha\) expression compared to the control at day 7. * $p < 0.05$}
\end{figure}
**Figure 3.** Histological analysis. FA group significantly decreased inflammatory root resorption rate compared to the control at day 28. *p < 0.05

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**P185**

**Citrus-Derived Eriocitrin has Beneficial Effects on Inflammation and Oxidative Stress**

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**Objectives**

The aim of this work was to investigate the anti-inflammatory properties of the citrus flavonoid eriocitrin (ERIOC) in *P. gingivalis*-stimulated macrophages and in a model of LPS-induced periodontal disease in mice. In addition, we assessed the antioxidative potential of ERIOC on keratinocytes and in gingival tissue of mice.

**Methods**

Enzyme-linked immunosorbent assay (ELISA) was performed to measure the production of pro-inflammatory cytokines interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF-α) by *P. gingivalis*-stimulated macrophages. The reactive oxygen species (ROS) production by gingival keratinocytes was measured using the fluorescent probe dichlorodihydrofluorescein diacetate (DCF-DA). In vivo, for 60 days 50 mice received either a standard diet, or a diet supplemented with 25 or 50 mg/kg body weight of eriocitrin. After 30 days of food supplementation, a solution containing LPS was injected into the gingival tissues three times per week for four weeks. Gingival tissue was collected, and oxidative damage was determined by measuring the malondialdehyde (MDA) content and anti-oxidative activity through the assessment of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Multiplex immunoassays were used to measure the interleukins IL-1β, TNF-α, and IL-10 in gingival tissue.

**Results**

Eriocitrin inhibited ROS production by gingival keratinocytes and decreased the levels of IL-6, IL-1β, and TNF-α secreted by macrophages challenged with *P. gingivalis*. The gingival tissue of ERIOC-supplemented mice presented levels of IL-1β and TNF-α lowest, while the production of IL-10 was highest when compared to control mice. Moreover, SOD, CAT and GPx activities were increased in the gingival tissues of mice that received ERIOC, while MDA content was reduced.

**Conclusions**

In conclusion, citrus-derived eriocitrin inhibits oxidative stress, improves the anti-oxidative defenses, and exerts potential anti-inflammatory activity.
**Blue Light Irradiation Influences Reparative Responses in Gingival Fibroblasts**

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**Objectives**
Photobiomodulation describes the application of light to influence cellular responses. Prior studies by ourselves have identified trends in reactive oxygen species (ROS) generation in gingival fibroblasts following irradiation with UV-free blue light, which affected cellular metabolic activities. Extending on these findings, the current study investigates whether blue light irradiation and elevated ROS production further influenced additional cellular reparative responses, including antioxidant, growth factor and extracellular matrix (ECM) gene expression.

**Methods**
Primary human gingival fibroblasts were serum-starved for 24h, prior to irradiation. Irradiances ranged from 3-90J/cm² generated from combinations of irradiance (mW) and treatment duration. At 6h and 24h post-irradiation, conditioned media from gingival fibroblasts were analysed for hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) protein levels. Gingival fibroblast expression analysis for our genes of interest (COL1A1, COL3A1, BGN, DCN, FN1, HAS2, HAS3, VTN and HGF), were performed by RT-qPCR. At 6h and 24h post-irradiation, gingival fibroblast cell lysates were also extracted for the analysis of GSH, NQO1 and KEAP1 antioxidants by Western blot analysis.

**Results**
Irradiances >3J/cm² induced significant dose-dependent increases in HGF gene transcription and protein levels, 24h post-irradiation. Irradiances >36J/cm² also induced significant increases in VEGF protein levels, 24h post-irradiation. Irradiances >36J/cm² induced significant upregulation in antioxidant gene expression (GSR, KEAP1 and NQO1), although these changes were not reflected by their protein levels. ECM genes were largely unaffected, although FN1, COL3A1 and HAS2 were downregulated by irradiances >36J/cm² at 6h and 24h post-irradiation.

**Conclusions**
There is emerging evidence for use of UV-free blue light to stimulate reparative responses in gingival fibroblasts. Blue light treatments induced significant increases in HGF and VEGF release by gingival fibroblasts. Non-significant changes in cellular antioxidants (GSH, NQO1, KEAP1), suggest that ROS generated during a single irradiation is transient and may not trigger *de novo* antioxidant transcription.

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**Retrospective Study of Minimally Invasive non-Surgical Treatment of Infraosseous Defects**

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**Objectives**
The aim of this study was to evaluate the effectiveness of minimally invasive non-surgical periodontal therapy (MINST) in the treatment of infrabony defects.

**Methods**
The use of minimally invasive procedures has recently been recommended for subgingival instrumentation during non-surgical periodontal therapy of periodontitis to minimize patient discomfort and maximize healing potential.

A retrospective analysis was performed on five infrabony defects treated in five patients with periodontitis. This treatment was based on the use of specific small-diameter ultrasonic inserts under low frequency ultrasound generator. The objectives are to eliminate subgingival biofilm and calculus, preserve tissue integrity and promote blood clot formation in the infrabony defect to allow healing.

All defects had a radiographic infraosseous component > 3mm and probing depths comprised between 6 and 9 mm. Clinical and radiographic data were available at baseline and 6 months after.

**Results**
After MINST, at 6 months, all patients showed healing of infrabony defects, decreased probing depths (of 3 at 5 mm) and an average clinical attachment gain of 3mm. All patients in this study exhibited healthy and reduced periodontium after MINST and thus did not benefit from periodontal surgery.

**Conclusions**
The minimally invasive approach should be the approach of choice for gingival instrumentation because data from the literature have shown a reduction in postoperative recession compared to a conventional approach.
Scl-Ab Influences Periodontal Tissues of Ovariectomized Rats During Orthodontic Movement

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Objectives The aim of this study was to evaluate the effect of a new drug based on an anti-sclerostin antibody (Scl-Ab) on ovariectomized rats' periodontal tissues during orthodontic tooth movement.

Methods A total of 48 rats were randomly distributed into 6 experimental groups (n=8/group): C (control), OM (orthodontic tooth movement), OVX (osteoporosis induction by ovariectomy surgery), OVX+OM, OVX+Scl-Ab (osteoporosis induction and Scl-Ab administration), and OVX+Scl-Ab+OM. All animals were submitted to OVX or Sham surgery (C and OM groups) 30 days before the day 0 (baseline). Scl-Ab administration started on baseline and OM on day 7. The body weight was recorded weekly, and after 21 days, all animals were euthanized by anesthetic overdose. All the animals were evaluated for alveolar bone volume (BV/TV) and bone density (BMD) by in vivo microcomputed tomography analysis (Micro-CT) after surgery and at baseline. After euthanasia, the maxillary was collected and separated into two hemimaxillae for BV/TV, BMD and tooth displacement analyses by Micro-CT. All data were tested for normality by the Shapiro-Wilk test. From that, One-Way ANOVA and Tukey’s post-hoc tests were used to compare the groups using a 5% significant level.

Results A significant increase in body weight and a decrease in womb weight were observed in all groups submitted to OVX. Furthermore, all ovariectomized animals presented a significant reduction in BMD and BV/TV parameters 30 days after the surgery. The OM and OVX+OM+Scl-Ab groups showed a tendency for higher tooth movement when compared to the OVX+OM group. In addition, the OVX+OM+Scl-Ab group showed an increase in BMD and BV/TV compared to OVX+OM and a decrease in these parameters compared to the OVX+Scl-Ab group.

Conclusions Our study showed that the Scl-Ab administration in ovariectomized rats reduces the harmful effects of osteoporosis on periodontal tissues submitted to orthodontic tooth movement.

Randomised Clinical Trial on the Short-Term Anti-Plaque Effect of Cymenol

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Objectives To analyse the in situ short-term anti-plaque effect of 0.1% cymenol mouthwashings, using Dentius Deep Plaque (DDP) software.

Methods Fifty non-smoking adults with good oral health status were randomly divided into two groups: subjects who rinsed three times daily with a placebo solution (placebo group) and those who rinsed three times daily with 0.1% cymenol (GingiLacer Encias Delicadas, Lacer S.A.; test group), both groups for four days (phase 1). All patients in both groups continued with 0.1% cymenol mouthwashings for a further four days (phase 2). During both phases, subjects refrained from performing any mechanical plaque removal techniques. The plaque levels of intraoral photographs were analyzed using the DDP software at three different times: T0 (baseline after professional cleaning), T1 (phase 1 ending), and T2 (phase 2 ending) obtaining percentage values of the visible plaque area and its growth rate per hour. The Student's test with Bonferroni correction (where necessary) was applied for intra- and inter-time comparisons.

Results At T0, the plaque area means were similar in both groups (placebo group = 5.68±3.53% vs test group = 5.57±3.57%). At T1, plaque levels were significantly lower in the test group compared to the placebo group at both T1 (29.80±13.97% vs 46.5% decrease from T1) and T2 (39.27±14.33% vs 59.24±16.90%, respectively). At T1, the plaque growth rate was significantly lower in the test group (0.20±0.07%; 46.5% decrease from T1). 0.1% Cymenol mouthwashings exhibit a short-term anti-plaque effect in situ, strongly conditioning the plaque growth rate even in clinical situations where high levels of dental plaque accumulation are present.
P193

Implication of Gremlin 1 in Periodontitis
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Objectives Gremlin-1 (Grem1) is an antagonist of bone morphogenic proteins (BMPs) and plays a critical role in embryogenesis, organ development, tissue differentiation, and bone homeostasis. It has been shown that Grem1 is involved in several inflammatory diseases such as osteoarthritis, organ fibrosis and cancer where its expression is upregulated. In this study, we aim to evaluate the expression of Grem1 in human gingival samples from periodontitis and to determine if Porphyromonas gingivalis infection could modulate its expression in periodontal cells.

Methods Gingival tissues from 25 patients (11 healthy and 14 in periodontitis group) were collected during open flap debridement (periodontitis group) or wisdom tooth extraction (healthy group). The expression of Grem1 and IL-1B was evaluated by RT-qPCR. In C57/BL6 mice, calvarial abscess was induced by P.gingivalis ATCC 33277 injection and markers expression by immunofluorescence. In vitro, oral epithelial cells, fibroblasts and osteoblasts were culture in specific media and infected for 24h with P.gingivalis (MOI=100). Grem1, IL-1B and BMP2 expression was evaluated in all cell types by RT-qPCR and immunofluorescence.

Results In clinical samples, the expression of both IL-1B and Grem1 was increased in periodontitis group confirming the inflammatory status of such tissues (3-fold and 1-fold respectively; p<0.05). Interestingly, in the calvarial abscess model, the injection of P.gingivalis increased significantly the expression of Grem1 especially within connective tissue. No specific effects were observed at the bone level. In vitro, the infection with P.gingivalis increased significantly Grem1 expression in oral epithelial cells but not in other tested cell types (5-fold; p<0.05). Interestingly, the expression of IL-1B and BMP2 was increased in all cell types (p<0.05).

Conclusions In this study, we established the potential involvement of Grem1 in periodontitis. Grem1 expression could be modulated by P.gingivalis infection specifically in oral epithelial cells. Future studies should decipher its precise implication during the establishment of periodontitis and its role in the inflammatory process.

P200

Toothbrush Disinfection With a Mouthwash Containing Chlorhexidine and Cetylpyridinium Chloride
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Objectives The aim of this study was to determine the antiseptic efficacy of a 0.05% chlorhexidine + 0.05% cetylpyridinium chloride mouthwash on toothbrushes used by orally healthy volunteers for two weeks.

Methods Toothbrushes (N=12) used 3 times/day for a total of 14 days were separated in two groups (control and treatment groups). Their heads were immersed for 2h in PBS (control group) or Perio Aid Active Control (treatment group). The total amount of microorganisms was recovered, and their number was calculated by cell culture, quantitative PCR, and viability PCR. Statistical differences were assessed with a Two-Way Mixed ANOVA, and subsequently with the Student’s t test.

Results The results showed a significantly lower number of live bacteria on the filaments of toothbrushes of the treatment group (mean ± CI95% of 4.58 ± 0.61 log10 viable bacteria/ml and 2.15 ± 1.42 log10 CFU/ml) than on those of the control group (6.49 ± 1.39 log10 viable bacteria/ml and 5.04 ± 0.93 log10 CFU/ml). However, no statistical differences in the total cell number (live + dead microorganisms) were observed when comparing the treatment (7.27 ± 1.09 log10 bacteria/ml) and the control (7.62 ± 0.64 log10 bacteria/ml) groups.

Conclusions This work proved that the disinfection of toothbrushes with a 0.05% chlorhexidine + 0.05% cetylpyridinium chloride mouthwash reduced the number of live microorganisms adhered to the filaments. This procedure would be advisable, especially for immunosuppressed people, to reduce the risk of both reinfection and cross-infection, caused by repeated exposure to pathogens and environmental microorganisms, as an alternative to a weekly or monthly replacement of contaminated toothbrushes.
National Network Group for Research and Innovation Within Oral Health


Objectives
In 2014, the Norwegian Ministry of Health and Care Services published a white paper on the status of oral health research in Norway, highlighting that there were too many small research groups in the country, focusing on too diverse subjects and that there was too little national and international cooperation in Norway.

Methods
A Government Action Plan was presented in 2017 stating that the Ministry should facilitate clinical research within oral health related to five prioritized fields: 1) practice-based research and innovation, 2) registry data, 3) interdisciplinary cooperation within research, 4) stronger national- and international cooperation, 5) strategic recruitment of researchers and improved conditions for research. On demand from the Ministry, a research network group with representatives from all oral health research institutions in Norway was established in 2018. The purpose was to follow-up the Action Plan by promoting dialogue and collaboration. The network should also be a meeting venue for updates, feedback and discussion, and should be able to assist the Ministry with advice related to oral health issues.

Results
The network group consists of 11 members representing all national oral health research institutions, 2 observers and 1 administrative secretary. Regular meetings with fruitful discussions have been arranged, and information has been collected from each institution on planned, ongoing and recently completed research activities within oral health. This information is being processed in an internet database. The establishment of the network group may be a valuable tool for promoting cooperation.

Conclusions
A strong goal of the network is to integrate clinical and translational research interests among oral health academic and research institutions, both nationally and internationally, and to break down barriers to inter-institutional and interdisciplinary clinical and translational research. We are getting there step by step.
Intra-Oral Scanner Investigation for Shovel-Shaped Incisor Analysis in Anthropology

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Objectives The latest development in 3D modeling has opened up new perspectives in dental morphology in anthropology.

In 2018, we published a new methodological approach to study modern human maxillary shovel-shaped central incisors, based on geometric morphometry (IU1) which is used as an indicator between population relationships.

Our method has been validated by significant statistical results on surfaces digitized by X-ray microtomography (micro-CT), recognized to be the standard for anthropological studies. It aims to improve the ASUDAS (Arizona State University Dental Anthropology System), currently the gold-standard method, by providing additional data.

A surface analysis doesn’t necessarily require the precision of such an imaging technique, which is difficult to access and very expensive.

In this preliminary research, we propose a new method for investigating the samples by making an optical impression via an intraoral scanner. The objectives are to see if the use of such an intra-oral scanner gives us sufficiently reliable and usable information.

Methods We scanned 31 modern humans UI1 (Europe) by 3 different imaging methods: X-ray microtomography, extra-oral laboratory scanner, intra-oral scanner. We applied our geometric morphometric protocol on each labial crown for each imaging method. To analyze the differences between the 3 scanning methods, a generalized analysis in Procruste and an analysis of variance using the ANOVA model and pairwise analysis were performed.

Results The accuracy and reproducibility of our morphometric approach are directly correlated to the accuracy of the scanning surfaces.

The intraoral scanner, which is a simple, fast, accessible, and inexpensive method of non-invasive 3D investigation of specimens, shows statistical results favorable to shovel ing analysis.

Conclusions A digital database would facilitate the archiving and better accessibility of information and decrease the risk of losing information over time, compared to conventional support (plaster models).
A New Surgical Guide for Implant Placement in Complete Edentulism
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Objectives The goal of an implant-based reconstruction is obtaining adequate aesthetic and function. In order to reduce post-treatment complications, ideal implant placement is necessary. The aim of this study was designing and fabricating a surgical guide for accurate positioning and angulation of dental implants in completely edentulous mandibular models and evaluate its effectiveness.

Methods The Designed surgical guide has the following components: the main body, working cylinder, and centralizer. The centralizer pin was placed in a hole created at the midline. The device rotates around the centralizer pin to be transferred to the other parts of the jaw. The cylinder has two holes on the lateral sides, corresponding with the implant surgical drill diameter, to create the guiding path. Drilling through the guiding path is possible after the adjustments. With the aid of the surgical guide, 16 dental implants were placed in two completely edentulous mandibular models. Afterwards, CBCT DICOM images were captured and analyzed by NNT Viewer software. Implant angle Deviations and distance from the planned angulation/position were compared. One-sample t-test was used for data analysis (at P<0.05 level of significance)

Results The mean angular deviation between the planned and placed implants was 3.31±1.2° and 0.97±0.56° for 0° (angulation relative to the vertical index at the midline) and 15° implants, respectively. The mean linear deviation between the planned and placed implants was 1.00±0.75 mm. Despite the differences being significant (P<0.05), they were clinically acceptable.

Conclusions The new designed surgical guide was efficient with little angular and linear deviation in the placed implants compared to the planned angulations/positions.
Focal Adhesion Formation of Primary Oral Fibroblast on TiO$_2$-Coated Titanium

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Objectives Optimal cell adhesion of the gingival fibroblasts to dental implants is essential in preventing peri-implant inflammation. The aim of this study was to discover if the bioactive TiO$_2$-coating on titanium alloy substrates is able to increase the cell adhesion of the human gingival fibroblasts (HGF).

Methods The study consisted of three differently produced titanium groups: 1) hydrothermally produced TiO$_2$-coating (HT), 2) TiO$_2$-coating made in sol (SOL) and 3) the control group. Primary HGF cells were initiated from gingival biopsies from patients having a wisdom tooth operation. HGF were cultivated on titanium discs for 2 and 24 hours to determine the initial attachment with confocal microscope. The cell spreading and adhesion protein signals on the bottom layer of cells were measured. In addition, expression of adhesion proteins vinculin, paxillin and focal adhesion kinase (FAK) were measured after three days of cultivation by using Western Blotting.

Results Higher protein levels of paxillin, vinculin and FAK were induced on both coated samples compared to non-coated samples. The difference was statistically significant ($p<0.05$) concerning expression of paxillin. The cell spreading was significantly larger on SOL samples after 2 and 24 hours when comparing to non-coated controls. The confocal analyses revealed significantly higher adhesion protein signals on both HT and SOL coated titanium compared to control group.

Conclusions This study showed, that both novel methods to produce a TiO$_2$-coating are able to increase HGF adhesion protein expression and cell spreading on titanium surface. Accordingly, the coatings have potential to ensure a more decent gingival attachment to titanium implant abutments.

Expression of focal adhesion proteins paxillin, vinculin and focal adhesion kinase (FAK) on non-coated, coating-made-in-sol and hydrothermally coated titanium. A.) Western blotting. B.) Signal levels of Paxillin, Vinculin and FAK balanced to GAPDH on coated and non-coated titanium surfaces after three days of cell culture. N= non-coated, Sol = coating-made-in-sol, HT= hydrothermal coating. Data represent mean and individual values. Significant p-values ($p < 0.05$) are marked in the figures.
Effect of directly in sol made and hydrothermally produced TiO$_2$-coatings on cell spreading after 2 and 24 hours. A.) Representative images of actin cytoskeleton and cell area after 2 and B.) 24 hours. C.) Quantifications of cell area after 2 and D.) 24 hours. N = non-coated, Sol = coating-made-in-sol, HT= hydrothermal coating. Data represent mean and individual values. Significant p-values (p < 0.05) are marked in the figures.
Effects of Experimental Setup on Dental-Implant Stability Testing in-Vitro

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**Objectives** In-vitro dental-implant-stability studies mainly place implants in Sawbones®. No standard protocol for experimental-setup and boundary-conditions is available. For development of an implant-stability clinical assessment tool, it is critical that the implant test model response be well calibrated, both theoretically and experimentally. This study aims to identify better criteria to test and interpret dental-implant-stability in-vitro.

**Methods** Experimental Setup: Branemark implants (NobelBiocare, Switzerland) (4 x 8.5, 10, 11.5, 13, 15, 18)mm were placed in artificial-bone models (Sawbones®) of different densities: hybrid, high-density, and low-density blocks using 2 different abutments.

Study of boundary-conditions: on-side (lateral) anchorage in a vise as opposed to a bottom-positioned anchorage of the Sawbones® (See image).

To calibrate the test model, natural-frequency-values measured by experimental modal analysis (EMA) and confirmed numerically by finite element analysis (FEA), for both boundary-conditions and experimental-setup. Natural-frequency measured by Osstell ISQ® and Periotest® were compared to EMA and FEA

**Results** The Osstell ISQ® and Periotest®PTV data were scattered and unable to predict the trend in boundary conditions.

Combined use of FEA and EMA confirmed that:
1. Test-models with bottom-anchorage had consistent results than side-anchored models.
2. Natural-frequency was not affected by change in implant length.
3. a 10% change in Sawbones density significantly affected the natural-frequency values.
4. EMA results correspond to FEA predictions with 20% marginal error.

**Conclusions** As boundary-conditions and experimental-setup play an important in dental-implant-testing, they should be taken into consideration when interpreting results of dental-implant-stability-testing in-vitro.
Individual and Implant Factors Associated With Peri-Implantitis: a Multilevel Analysis
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Universidade Paulista, São Paulo, Brazil

Objectives The aim of this study was to perform a multilevel analysis of the factors associated with peri-implantitis (PI) in patients rehabilitated with implants at Universidade Paulista (UNIP).

Methods A total of 171 patients with 668 implants who had been in function for at least 5 years were selected and clinical and radiographic parameters, systemic factors and implant characteristics were evaluated to determine peri-implant health.

Results Quality of life was assessed using the Oral Health Impact Profile Index (OHPI-14). One hundred and thirty implants (19.5%) had PI, with 57.8% in patients with gingival bleeding (GB) >20% and 37.4% in those with BOP<20%. Patients over 50 years of age and probing depth (PD) > 3mm were more likely to have PI. Number of implants and clinical attachment level (CAL) were also associated with PI. Within the implant factor, GB, PD, the distance from the gingival margin to the platform (GM-IP) and the type of connection were associated with PI. Implants with PS > 3 mm had an adjusted OR 9.75 times more likely to have PI, and those with GM-IP distance from the faces smaller than 0 were 7.42 times more likely to have PI. In the multilevel model, patients with full-mouth PD > 3 mm were 2.45 times more likely to have PI.

Conclusions Quality of life was not affected by PI. Thus, PD, both at the implant and patient level, and the GM-IP of implants are risk factors for PI. However, due to the low prevalence of the disease, there was no negative impact on the patients' quality of life.

The "Implant Protection Plan": Preventing Implant Diseases Focusing on Compliance
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Objectives The prevention of peri-implant diseases is essential to ensure the success of a rehabilitation. Faced with an increasing number of dental implants placed, the relevance of peri-implantitis makes it an important problem for the dentist and hygienist. The purpose of this work is to describe the effectiveness of an implant maintenance plan called "Implant Protection Plan" in the prevention of related diseases.

Methods A group of experts in implantology, periodontology, prevention and dental hygiene was involved in a workshop with the aim of evaluating their clinical experiences in the field of prevention and treatment of peri-implantitis. Patient behaviors after the delivery of rehabilitations, advice and prescriptions normally given, patients' adherence to the maintenance plan, home and professional oral hygiene programs and the effectiveness of mechanical hygiene tools were analyzed. In addition, a questionnaire was distributed to dentists and dental hygienists outside the working group.

Results 3 different clinical forms have been developed, useful for motivating the patient and monitoring the effectiveness of the prescriptions given at the time of the visit. It was possible to monitor the effectiveness of the mechanical hygiene tools, accurately identifying when to replace them or evaluate an alternative. It was possible to minimize the number of recall appointments canceled or canceled due to forgetfulness by the patient, ensuring continuity in maintenance. Patients felt involved in the maintenance plan, better understanding the importance of the program.

Conclusions From the clinical results evaluated, it is possible to state that an implant maintenance plan such as Implant Protection Plan can represent a successful tool for the prevention of peri-implantitis. Too often, in fact, even the patient who receives excellent implant rehabilitation does not receive an adequate program and exposes himself to avoidable risks. Some adaptations to dentists' maintenance plan combined with support for verifying the efficacy of prescriptions is certainly an optimal clinical behavior for the success of therapies.
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Note:
STATIC BACTERIAL LEAKAGE AND DYNAMIC SEALING ABILITY OF IMPLANT CONNECTIONS

Morena Petrini1, Antonio Scarano2, Tatiane C. Dotta3, Niloofar Etemadi4, Marzieh Ramezani Farani8, Giovanna Iezzi1, Felice Lorusso2, Adriano Piattelli5,6, Luca Comuzzi7, Simonetta D’Ercole1
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Objectives The objective of this study was to test the bacterial leakage and the sealing ability of three different implant-abutment connections.

Methods 90 implants were distinguished into External Hexagon (EH), Internal Hexagon (IH), and Cone Morse (CM).

For the bacterial leakage in static conditions, 60 implants were inserted on sterile special vinyl supports, and the platforms were inoculated with 1 μL of standardized broth cultures. Each group was distinguished into two sub-groups, based on the bacterium that was used for the inoculation: Streptococcus oralis (SO) and Pseudomonas aeruginosa (PA). All abutments were connected to each implant, put in sterile tubes with caps, containing broth culture, and observed daily for 14 days. Samples were checked daily and results were recorded dichotomously as: contaminated (cloudy broth)/no contaminated (clear broth).

For the evaluation of the sealing ability in dynamic conditions, 30 implants (10 of each group) with the connected abutments, were mounted inside a customized stub equipped with internal liquid storage containing a solution of water and toluidine blue and underwent 1 × 10⁶ loading cycles at 30° inclination, with 20 - 100 N/cm load, by using a Lloyd Universal testing machine. Results were recorded dichotomously as: infiltrated (presence of toluidine blue inside the implant platform)/no infiltrated. The ANOVA analysis and LSD were calculated, in order to find significativity among groups (p< 0.05).

The random observation of 10 implants at the scanning electron microscope, confirmed the complete fitting of the abutments of the fixtures.

Results CM showed a significantly lower bacterial contamination, the 20% of all implants (0 % SO and 40% PA), with respect to EH 45% (30% SO and 60% PA), and IH 55% (40% SO and 70% PA). The dynamic tests, showed no infiltrations on CM and IH, contrary to EH which showed 30% of infiltration.

Conclusions In conclusion, CM showed a higher sealing ability and lower bacterial leakage on both static and dynamic tests.

STUDY DESIGN: Bacterial leakage in static conditions (A-D). A) Bacterial inoculation; B) Abutment connection; C) Implants submersed with broth culture; D) Implant contaminated (left)/Implant non contaminated (right). Dynamic sealing test (E-H). E) Implant/abutment connected and fixed at 30°; F) Implant/abutment submersed in the solution of toluidine blue in the Lloyd Machine. G) Implant/abutment subjected to the 30° loading cycles. H) Paper point used to verify the presence of toluidine blue, inside the implant platform.
An Operatory Dental Implant Surgical Guide for Partial Edentulism

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Objectives With the increasing demand for dental implants, related complications are increasing as well. This is particularly significant in partial edentulism, for it may have an undesirable effect on the adjacent teeth. Several tools and surgical guides have been developed to prevent these complications. The objective of this study was to design, fabricate and assess the efficacy of a novel surgical guide for dental implants in partially edentulous patients.

Methods The designed surgical guide consists of one graded rocket and 8 accessories (4 pairs) with diameters of 6,7,8 and 9 millimeters and the height of 6mm. Each of the accessory pairs have two passage holes (for 2 and 2.8 mm drills). Efficacy was measured by placing 15 implants in identical partially edentulous jaw models. Postoperative CBCT was obtained and used for analyzing the following three measurements: implant-tooth mesiodistal distance, inter-implant mesiodistal distance, and inter-implant angulation. Data analysis was done by One sample T-test. (α=0.005)

Results One Sample T-test revealed statistically significant difference between the planned and actual values in the implant-tooth mesiodistal distance (P<0.001) and inter-implant distance (P=0.005). However, no statistically significant difference was found in implant angulation(P=0.073). The mean implant-tooth mesiodistal distance, inter-implant mesiodistal distance, and inter-implant angulation were 0.29±0.19 mm, 0.03±0.17 mm, and 3.88±4.20°, respectively.

Conclusions Clinically acceptable mean values were achieved in this study. This novel surgical guide showed reliable positional accuracy in achieving optimal inter-implant angulation, inter-implant mesiodistal distance and tooth-implant mesiodistal distance, providing a simple and straightforward option for the clinician.

P214

Pectin’s Antimicrobial Effect Against Bacteria Associated With Endodontic Infections.

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Objectives To examine the antimicrobial effect of pectin on two bacterial species that are commonly associated with root canal infection and pulp necrosis, individually and within a dual-species biofilm.

Methods The antimicrobial effect of pectin was tested against E. faecalis and F. nucleatum. In preliminary experiments, three concentrations (1%, 2%, and 4%) of low methoxyl commercial citrus pectin LM (pectin CU701) (Herbstreith & Fox, Germany), were added to brain heart infusion broth medium (BHI) to grow E. faecalis. Five-hour growth curves were obtained by measuring the OD of bacterial suspensions every hour. A dual-species (E. faecalis and F. nucleatum) biofilm was then developed using the minimum biofilm eradication concentration (MBEC ) assay kit (Innovotech, Ca) to test the minimum biofilm eradication concentration of pectin. Based on initial findings, 2%, 4% and 8% pectin were prepared, and bacterial abundance was calculated using viable counting methods under 24h aerobic (BHI agar) and anaerobic (Fastidious Anaerobic agar) conditions. The results showed that pectin had a concentration-dependent inhibitory effect on E. faecalis growth curve in comparison to the negative control group (no antimicrobial treatment). However, pectin showed a different effect on the growth behaviour of E. faecalis and F. nucleatum when grown in a mixed biofilm. The higher concentrations of pectin (4% & 8%) seem to enhance bacterial growth by 2 folds and one and a half fold respectively in comparison to control, whereas growth reduction by half fold occurred at 2% pectin.

Conclusions Within a dual-species biofilm, a 2% pectin medium reduced bacterial growth, which was in contrast to single strain bacterial growth. Further work is being carried out to investigate the impact of pectin on biofilm composition within a more complex co-culture biofilm model.
P218

The Oral Cavity: a Potential Reservoir for Diabetic-Foot Ulcer Infections

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Objectives Oral carriage of Staphylococcus aureus is a potential risk for diabetic foot ulcer infections (DFUs) in patients with Type 2 Diabetes (T2D), and carriage is higher in periodontal disease patients, a condition more prevalent in T2D.

Methods Staphylococcal species were recovered from anatomical sites (oral cavity, nares, periodontal pockets, finger, toe, ulcer) of diabetic patients with and without diabetic foot ulcers (DFUs) using selective chromogenic media. Staphylococcus aureus (N=263) and S. epidermidis (N=287) isolates from distinct sites were investigated by Illumina-MiSeq (The Netherlands) short-read whole-genome sequencing and whole-genome multilocus sequence typing (wgMLST) to determine genetic relatedness.

Results Staphylococcus aureus was recovered from the mouths of 20/31 (64.5%) and ulcers of 9/31 (29.0%) patients with DFUs. Eight patients yielded S. aureus from both sites. Closely related isolates (≤24 wgMLST allelic differences) were recovered from multiple sites in four patients, two of whom yielded closely related oral and ulcer isolates. Staphylococcus epidermidis was recovered from the mouths of 17/31 (54.8%) and ulcers of 6/31 (19.4%) DFU patients. Five patients harboured S. epidermidis at both sites. Closely related S. epidermidis were recovered from multiple sites of five patients with DFUs. Closely related pairs of oral and ulcer isolates were not detected.

The prevalence of staphylococci was also investigated from the same sites in patients without DFUs. The prevalence of oral S. aureus and S. epidermidis in these patients was significantly lower (23/71 [32.4%], (p=0.0139) and 26/71 [36.6%], (p=0.0014)), respectively. Closely related S. aureus and S. epidermidis strains were detected from multiple sites in six and four patients, respectively.

Conclusions Study findings showed the prevalence of oral S. aureus and S. epidermidis is significantly higher in patients with DFUs. The detection of closely related oral and ulcer S. aureus isolates in patients with DFUs provides evidence that the mouth may be a reservoir for DFUs.

P219

Development of an Iridium Complex as an Antimicrobial Photodynamic Therapy

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Objectives Photodynamic therapy (PDT) is a process where, upon illumination, a photosensitiser becomes toxic to either cells or bacteria often through the generation of reactive oxygen free radicals. Such technology has been used clinically for the treatment of periodontal disease but with unproven effectiveness. This may be due to the ability of periodontal pathogens such as Porphyromonas gingivalis to avoid antibacterial agents and host defences by invading host cells.

We hypothesise that by developing new photosensitisers which can localise to and kill bacteria within host cells that this may improve the efficiency of antibacterial PDT.

Methods H357 oral epithelial cells were incubated with a novel mononuclear iridium (III) complex for 2 hours followed by illumination with a 405nm laser at 20mW/cm² for 3 min. Treated control samples were kept in the dark. Cell viability was then assessed using an MTT assay. Toxicity against P. gingivalis in suspension and 5 day biofilms was determined using similar protocols followed by viable plating. Confocal structured illumination and electron microscopy was also used to visualise the localisation of the photosensitiser. Finally, an antibiotic protection assay was used to determine the effect of the PDT on intracellular organisms.

Results Oral epithelial cells exhibited >90% viability following exposure to photosensitiser concentrations up to 1mM and laser light. PDT resulted in >99% killing of both planktonic and intracellular P. gingivalis (2nM) although biofilms were less sensitive (60% reduction). Microscopy indicated that the sensitisier passes through both host cell and bacterial membranes, the latter being damaged by the PDT.

Conclusions We have shown that a novel iridium complex can be used as part of an antimicrobial PDT strategy to effectively target P. gingivalis including those present within host cells. Such information is of importance in the development of new clinical treatments for periodontal disease.
An in Vitro 3D Oral Mucosa Model (3DOMM) for Infection Studies
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1Peninsula School of Dentistry, University of Plymouth, Plymouth, United Kingdom, 2School of Biomedical Sciences, University of Plymouth, Plymouth, Devon, United Kingdom

Objectives To assess the efficacy of primary oral keratinocytes (P3D oral mucosa model, P3DOMM) and immortalised HaCaT (3D oral mucosa model 3DOMM) cells to model single and dual infections of C. albicans and S. aureus for studying tissue colonisation and invasion, and subsequent pro-inflammatory cytokine production.

Methods Models were infected with clinically relevant strains of S. aureus (IRAS ref. 208291) and/or the C. albicans type strain (SC5314) and subsequently evaluated for tissue invasion, cell pathology and pro-inflammatory cytokine production by histology, SEM, LDH production and ELISA. Different combinations of patient samples, and type strains, were added to HaCaT cells to compare isolates obtained from patients with single and dual colonisation of S. aureus and C. albicans for 24h prior to ELISA analyses for IL-6 and IL-8. Statistical analyses confirmed parametric vs. non-parametric data; p-values < 0.05 indicated statistical significance.

Results HaCaT cells alone and in 3D mucosal models produced pro-inflammatory cytokines in response to infection. For HaCaT cells, 3DOMMs, and P3DOMMs, a significant increase in pro-inflammatory cytokine production (IL-6; IL-8 and IL-8; IL-6, respectively) was observed between the unstimulated control and models stimulated with C. albicans or S. aureus, and dual infection. 3DOMMs only were capable of increasing both IL-6 and IL-8 in response to live infective stimuli. This means that despite the high levels of constitutive pro-inflammatory cytokine production identified, it is still possible to measure upregulation in response to live infection.

Conclusions 3DOMM produced IL-6 and IL-8 pro-inflammatory cytokines in response to single and dual infections of C. albicans and S. aureus, as well as indicating its suitability to model microbial colonisation in a robust, reproducible, manner.

Culture of Methanogenic Archaea From Necrotic Dental Pulp
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Objectives To successfully cultivate methanogenic archaea from necrotic dental pulp in order to confirm their presence and better understand their involvement in endodontic pathologies.

Methods Teeth extracted for medical reasons were collected after approval by the ethics committee. The teeth were disinfected and sectioned and the necrotic pulp was removed and cultured under specific conditions for the growth of methanogenic archaea. Controls consisted of pulp from healthy wisdom teeth.

Results Methanobrevibacter oralis and a very recently discovered and poorly described species Methanobrevibacter massiliense were cultivated from necrotic dental pulps.

Conclusions Methanogenic archaea are clearly involved in endodontic pathologies, which could have consequences on prescription habits and endodontic treatments.
**Impact of Oral Anticoagulants on PRF Efficacy**

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**Objectives**

The use of PRF in dental surgery is now used, according to the surgical teams, on a daily basis and in different formats (platelet-rich fibrin (A-PRF) or injected PRF (I-PRF)). This platelet concentrate would release in situ platelet growth factors, improve healing, promote periodontal regeneration but also provide an analgesic effect.

However, there are no limitations on the use of PRF in the literature, regardless of the type of pathology or drug interactions.

Long-term prescription of oral anticoagulants in patients over 70 years of age is common. These treatments aim to slow down coagulation through a direct or indirect inhibitory action on coagulation factors. We hypothesize that their administration modifies the structure of PRF and its properties in these patients, which could limit its use.

The objective of this study was to analyze, in vitro, the structure of PRF clots and the release of growth factors in patients on anticoagulants.

**Methods**

The three-dimensional structure was analyzed by scanning electron microscopy. Growth factor assays were performed in the growing medium, by ELISA or Luminex, to visualize the levels of the vascular endothelial growth factor (VEGF) and the insulin-like growth factor-1 (IGF). Serotonin is determined by high performance liquid chromatography (HPLC).

**Results**

We will present the preliminary results which will be compared with those obtained in healthy subjects but also for patients before and after the start of their treatment.

**Conclusions**

This original study could challenge the paradigms for the use of this blood derivative in patients requiring long-term anticoagulant treatment.

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**Crocus Sativus Extract, a Promising Oral Ingredient for Periodontitis Prevention.**

christophe capallere, Imane Garcia, Marianne Arcioni, Celine Meyrignac, Christelle Plaza, Justine Cotton, Isabelle Imbert

Ashland, Sophia Antipolis, France

**Objectives**

Crocus Sativus (C. sativus) is a widely known plant, especially for its antioxidant properties. Periodontitis, a chronic inflammation is characterized by gum bleeding, gums recession and even tooth loss. This oral disease is initiated with oral biofilm dysbiosis with Porphyromonas gingivalis (P. gingivalis) having the most important role in periodontitis development. The aim of the present study was to investigate the effects of C. sativus flower extract on some of the virulence factors of P. gingivalis but also on the host immune system and epithelium barrier function.

**Methods**

Inhibitory potential of C. sativus flower extract was evaluated against gingipains of P. gingivalis by using chromogenic peptides as substrates. Hemolysis inhibition assays were performed on human red blood cells in contact with P. gingivalis with or without of C. sativus flower extract. Gingival human keratinocytes were treated with planktonic cultures of P. gingivalis with or without C. sativus flower extract and bacterial adhesion was determined by immunostaining. C. sativus flower extract effect on macrophages polarization was evaluated in vitro on Human PBMC derived macrophages by measuring CD14 and CD163 expression levels.

**Results**

Gingipain activity decreased by 74 and 85% in two independent assays following 1% C. sativus flower extract treatment. Then, C. Sativus flower extract at 1% also showed significant inhibitory activity (48% and 78%) against P. gingivalis induced hemolysis. Finally, C. sativus flower extract induced an increase in cell proportion of type 2 macrophages expressing both CD14 and CD163 on their surface.

**Conclusions**

C. sativus flower extract showed significant effects against important pathogenic aspects of P. gingivalis. It also promoted anti-inflammatory macrophages polarization. C. sativus flower extract may be a promising candidate for periodontitis prevention and gum reinforcement.
**P225**

*Whitening Effect of Chewing Gums Mastication for 30” or 5’*

Pier Francesco Porciani, Caterina Perra, Simone Grandini

*University of Siena, Italy, Firenze, Italy*

**Objectives** To evaluate the whitening effect of two chewing gums added with E132 and/or spirulina after 30” or 5’ of mastication.

**Methods** Subjects meeting inclusion criteria entered the trial and received a personal silicone mask with a calibrated hole at one upper incisor to apply the tip of a portable dental colorimeter (Vita® Easyshade Advance 4.0). Two chewing gums were tested, Gum1 (1.4g/piece) was added with E132 and spirulina, and Gum2 (1g/piece) with only spirulina. They were sugarless and provided by the manufacturer (Perfetti Van Melle S.p.A.). The tooth color was scored by the Vita® Easyshade Advance 4.0 with the CIELAB system and two whitening indexes WIO and WI_D were calculated. The color of teeth was scored before the assumption of one piece of chewing gum and after 30” or 5’ of mastication. Statistical analysis was performed intra-group by paired T-test and inter-group by ANOVA.

**Results** 196 subjects were enrolled, 165 joined and completed the trial. After 30” the differences for WIO and WI_D were respectively 1.85 and 1.45 for Gum1 and 1.85 and 1.29 for Gum2, after 5’ they were respectively 1.21 and 1.20 for Gum 1 and 1.53 and 1.01 for Gum 2. Both chewing gums significantly (p<0.05) increased the WIO and WI_D either after 30” and 5’ without significant difference between them and the period of mastication. The difference in WI_D index was found to overcome the perceptual thresholds of 0.94 for inexperienced observers with both chewing gums and with both periods of mastication and no subjects reported side effects.

**Conclusions** The observations reported in this study validate the optical instant whitening effect of both these new chewing gums after 30” or 5’ of mastication as approximated by the WIO and WI_D indexes with similar results to those reported in previous studies conducted with the same chewing gums for 2’30”.

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**P226**

*Effect of Zinc Lactate Added Candies on Oral VSC*

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¹University of Siena Italy, Firenze, Italy, ²University of Siena Italy, Firenze, Italy

**Objectives** To assess the efficacy of sugar-free candies containing zinc lactate vs. placebo on oral volatile sulfur-containing compounds (VSC).

**Methods** This trial was cross-over, randomized, double-blinded, single-center. To join, subjects had to have at least 24 teeth, no oral or systemic diseases and no dentures. Eligible participants had to avoid professional oral hygiene and drugs for two weeks, to be not menstruating and for six hours before the test, to avoid brushing their teeth, smoking, assuming alcohol, coffee, tea, onion, garlic, licorice. They had to score a level of VSC ≥75 ppb at the basal measurement. The test candy (2g) contained zinc lactate delivering 0.51mg of zinc, the placebo was identical without zinc. The OralChroma2© device was utilized to measure VSC in the oral air. The levels were recorded at baseline, after sucking one candy, after 30 minutes and 1 hour. Data were analyzed intra-group with paired T-test and between the groups with unpaired T-test (α=0.05).

**Results** 15 subjects (23.9±10.3y.o.) completed the trial. None reported problems linked to the assumption of zinc lactate. In the test group the mean reduction from baseline at the end of candies sucking was 69% (p<0.001), after 30 minutes 44% (p<0.05), after 1 hour 24% (p<0.05). In the control group the mean reduction from baseline at the end of candies sucking was 39% (p<0.001), after 30 minutes 13% (p<0.01), after 1 hour, it was increased by 2% (p=NS). The test candy showed a better performance versus placebo with a significant difference after sucking (p<0.01), after 30 minutes (p<0.05), after 1 hour (p<0.001).

**Conclusions** The assumption of candies containing zinc lactate can statistically significantly reduce the oral VSC levels from baseline immediately and over 1 hour and significantly more than placebo at any time.
P227
Quality of Life in Head and Neck Cancer Patients
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Objectives To elucidate general dental professionals’ clinical management of individuals with oral dryness and awareness of the importance of saliva

Methods An electronic pilot-tested survey consisting of 32 questions was sent to 2445 dental professionals (DP) working in general dental care in three counties in Sweden. The questionnaire included questions about the clinical management of individuals with oral dryness, education and experience of dental care for individuals with oral dryness, and self-assessment questions regarding knowledge about saliva.

Results The questionnaire was answered by 495 DP, 51% dentists and 49% dental hygienists. 91.2% had worked ≥ 2 years in their profession and 51.7% > 10 years. 84.5% had completed their undergraduate education in Sweden and had gained their knowledge regarding saliva and oral dryness during undergraduate education (95.4%). DPs encountered individuals with oral dryness several times a week (37%) or less often. More dental hygienists (60%) than dentists (44%) asked the age group 18-23 years about oral dryness. The three most frequent recommendations were extra fluoride (61%), saliva stimulating products (59%), and rinse with water (49%). 44% rarely contacted a doctor for medication exchange and 32% never did so. The two main reasons for measuring the stimulated salivary secretion rate were clinical signs of oral dryness (53%) and answers from the medical history questions (41%). 91% of the dental hygienists and 50% of the dentists stated that they had measured patients’ salivary secretion rate. Both professions (75%) wanted more education regarding the importance of saliva for oral health.

Conclusions DPs have relatively good knowledge of what causes oral dryness and its complications. Dental hygienists seem to take care of individuals with oral dryness to a greater extent than dentists. Dental hygienists rated their knowledge about the care and treatment of individuals with oral dryness higher than dentists.
P229
Comparative Assessment of Oral Health Risk Factors Related to Diabetes Type 2 – an Analytical Cross-Sectional Study
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Objectives Diabetes mellitus is a global health problem and patients with diabetes are at risk of developing oral health complications. This study aimed to assess and compare the socio-demographic factors, and dietary and oral health behaviours among diabetic and non-diabetic individuals.
Methods Ethical approval was obtained from the Office for Research Ethics Committees (08/H0702/54). In this study, a total of 182 participants either with diabetes type 2 (D) or non-diabetes (ND) (n=91 each group) were recruited and requested to complete a prior-validated questionnaire with 33-items related to socio-demographic factors, oral health and dietary habits. The descriptive and inferential statistical analysis were carried out using SPSS v.21. Chi-square test of proportion and associations were also performed at a 95% confidence interval (p<0.05). An analytical
Results There were male (n=92) and female (n=89) participants. 38% of diabetes participants were British Asian while only 12% of non-diabetes are different ethnicity. Majority of these participants had no dental insurance (98%). 31.8% were on benefits and 39.5% of diabetics were retired whilst 46% of non-diabetes are working full-time. Juice consumption was avoided by 34% of participants with diabetes and 27% of non-diabetes. Daily snack consumption was similar between the groups (D=34%, ND= 40%). Toothbrushing twice a day was slightly less in participants with diabetes (68%) when compared to non-diabetes (78%). Half of the participants failed to carry out interdental cleaning (D=52% and ND=47%) and 38.5% of the diabetes group used mouthwash occasionally whilst 30% of non-diabetes had it twice a day. Regular annual dental check-ups were observed among diabetes participants (30%) while compared to non-diabetes (22%). Statistical analysis failed to show any association between oral health behaviours between the groups except the presence of dental fixed prosthesis showed statistical differences (p>0.05). There was a significant difference in juice and sweetened juice consumption between the groups (p<0.05).
Conclusions In conclusion, factors related to socio-demographic and oral health behaviours were similar between diabetics and nondiabetics. However, diet habits and the presence of fixed prosthesis demonstrated significant differences between these groups.

P231
In Vitro Assessment of Manual Toothbrush Wear
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Objectives Due to mechanical interactions during brushing, toothbrushes are subject to a certain amount of wear. However, there is no standard method for evaluating toothbrushes under conditions of use. Therefore, tests are needed to assess wear resistance of bristles or bundles as well as the entire head design. In addition, it is of interest to evaluate how different brushing techniques or parameters affect wear. Aim of this study was to develop a reliable and standardised method to assess the degree of manual toothbrush wear and to investigate the influence of brushing movements.
Methods A commercially available brushing simulator (JWE GmbH, Germany) was used and further developed. Standard toothbrushes (Dr. Best Interdent Aktiv, GSK) were tested by brushing artificial denture tooth rows under load (4N) for up to 80,000 cycles. Tests were performed comparing different brushing movements (linear, circular and combined) with toothpaste slurry or water. Before and after the tests, the bristle field of the toothbrush head was photographed. The bristle field area [mm²] was measured using a digital software (ImageJ) and statistically analyzed (ANOVA, Bonferroni-test).
Results It could be shown that linear reciprocating movements caused 20% higher wear in comparison to circular and 7% higher wear in comparison to combined movements (p<0.004). At brushing with linear movements, the addition of toothpaste slurry caused 7% higher wear compared to brushing with water (p<0.0001).
Conclusions The developed methodology is suitable to reproducibly evaluate toothbrush wear and to provide quantitative data by comparing toothbrushes at various stages of splaying. Therefore, this method seems to be suitable for R&D, quality control, standardization and to support claims. As a clinically relevant result, it can be noted that linear brushing, i.e., non-recommended "scrubbing", causes more wear than circular or combined brushing.
P232
Characterization of Phosphate Speciation in Saliva and Oral Care Products
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Objectives
The aim was to investigate the speciation of typically sourced soluble phosphates from saliva, oral care products, and phosphate glasses. The effect of the alkaline phosphatase on the oral ecosystem of the phosphates was monitored using $^{31}$P NMR spectroscopy.

Methods
The Tris solution was used as a medium to dissolve selected type of phosphates. Three concentrations of total phosphates 30 ppm, 100 ppm, and 300 ppm mimicking their amount in toothpaste and the remineralizing agents. The following phosphates were selected: $\text{KH}_2\text{PO}_4$, $\text{Na}_2\text{HPO}_4$, $\text{Na}_2\text{FPO}_3$, $(\text{NaPO}_3)_6$, $\text{Ca(H}_2\text{PO}_4)\cdot\text{H}_2\text{O}$ and $\text{K}_2\text{HPO}_4\cdot3\text{H}_2\text{O}$ in addition to the buffer solutions of artificial saliva at pH 4, pH 7, and PBS. Solution-/solid-state $^{31}$P NMR spectroscopy, and X-ray diffraction were used to characterize the dissolved state of phosphates available in oral health products and other phosphate sources including phosphate glasses in the oral environment with and without alkaline phosphatase.

Results
The results indicate that various types of soluble phosphates exhibited different $^{31}$P NMR spectra. The signals observed in the $^{31}$P NMR spectra fall into three distinct regions. The chemical shifts of $^{31}$P NMR spectra were affected by the pH. Enzymatic hydrolysis of condensed phosphate groups in solution by alkaline phosphatase was found to occur.

Conclusions
Qualitative and quantitative evaluation of the P species in phosphates used in oral care products were done. These findings are important for the development of the new phosphates-containing remineralizing products or generation of bone grafts, including the for the periodontium repair.

P233
Effect of LLLT on Periodontal Obese Patients
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Objectives
Periodontitis has been reported to relate to metabolic syndrome traits such as obesity, blood pressure, diabetes. The aim of this study is to assess the histological effectiveness of a low-level laser therapy (LLLT) with respect to the acceleration of bone regeneration after surgical treatment of intrabony defects of patients with morbid obesity.

Methods
Twelve patients with intrabony defects, aged between 30-55 with morbid obesity, non-smokers, were randomly divided in two groups. Each patient had at last one periodontal defect treated by bone allograft. The test group received a postsurgical treatment with LLLT. The equipment used was OsseoPulse AM300 (extra oral light emitting diode), at an intensity of 20mW/cm$^2$ for 20 minutes per day for 21 consecutive days. The control group received no treatment with LLLT. The bone formation was evaluated in both groups at baseline and 6 months postoperative by the means of a biopsy of tissue followed by a histological analysis.

Results
The histological study of the control sample harvested 6 months postoperative showed of irregular fragments of bone in loose fibrous connective tissue. The diagnosis of this tissue sample is evaluated to be a vital lamellar bone with chronic inflammation of the narrow spaces.

The histological study of the test sample at 6 months after regeneration showed bone formation without inflammatory cells and occasional nonviable bone consistent with regenerating bone. No evidence of the grafted material is present.

Conclusions
With all the limitations regarding the number of patients and the follow up period, the histological analysis showed that the intrabony defects regenerated by bone allograft and treated with LLLT produces more significantly bone that the non-treated defects. Histological study suggests that in 6 months there is new bone formation in the defects treated with LLLT.
Data Collection Using ‘Software as a Service’ in Oral Health Surveys.

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Objectives Data collection methods and storage are an essential component of data management and reproducible research. Accurate data collection minimises potential problems for open science sharing of data and enhances methodological robustness by facilitating computational reproducibility. Software as a Service (SaaS) is a method of providing access to applications via the internet. Selection of suitable SaaS applications depends on multiple factors including platform options, design and system features. Protocols to apply SaaS applications were developed for research aimed at investigating the oral health and dietary pattern of elite athletes in Ireland.

Methods KoboToolbox and FoodBook24 are SaaS tools that can record data offline and provide encrypted transfer to cloud storage on databases using Amazon Web Services. KoboToolbox is a SaaS built on the XForm/ODK standard that provides field data collection, initial analysis and visualisation. FoodBook24 is a dietary assessment application that comprises 24-hour dietary recall (24HR) and a food frequency questionnaire. For this survey KoboToolbox was used to create a bespoke supplementary dietary behaviours and lifestyle questionnaire, and a clinical examination record based on a modified WHO oral health assessment.

Results Customised oral health survey forms were designed using KoboToolbox to collect data from elite athletes via a clinical examination. FoodBook24 was provided for automated self-completed of 24HR on three different occasions and produce a nutrient report. The advantages over paper-based data collection in oral health survey projects include rapid recording on different platforms, incorporating skip logic and validation criteria, reduced errors in data transfer, a clear record of the data including any changes in form design and version control.

Conclusions While there is a learning process and training is required to use these digital tools effectively, they provide a complete hosted solution service for designing, receiving and processing form submissions making the research process more effective, easier and reproducible.

Segmentation of Lesions in 3D CBCTs With Self-Configuring Deep Learning

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Objectives The purpose of this study was to develop a machine learning algorithm for the detection and segmentation of radiolucent lesions of the jaws using nnU-Net, a self-configuring method for biomedical image segmentation.

Methods We used 105 cone beam CT (CBCT) volumes with radiolucent bone lesions sourced from an Indian subpopulation with a mean [sd, min, max] age of 46.1 [11.9, 11, 90] (66.7% males and 33.3% females). The lesions were segmented using a semi-automated segmentation technique by two independent specialists. We used nnU-Net, a deep learning-based segmentation model, which configures preprocessing, network architecture, training and post-processing itself. Configurations were among others determined by training four different network architectures in a 5-fold cross-validation scheme, namely 2D U-Net, 3D full-resolution U-Net, 3D low-resolution U-Net, and a cascade of 3D U-Nets performing consequently on low and full-resolution CBCTs. The evaluation was performed for all independent models and their ensembles.

Results Based on the average Dice scores [95% Confidence Interval] the nnU-Net selected the ensemble of 3D full-resolution U-Net and the cascade of 3D U-Nets as best performing configuration (0.69 [0.64; 1.0]), although results without statistical significant difference (p=0.9/t-test) were achieved by the ensemble of 3D full-resolution U-Net and 3D low-resolution U-Net (0.69 [0.63; 1.0]).

Conclusions Self-configuring deep-learning can be successfully applied for the segmentation of radiolucent lesions of the jaws.
Importance of Fluoride Bio-Accessibility for Oral Health

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Objectives Although fluoride, at low levels, has a key role in the prevention of dental caries, excessive systematic intake can lead to the development of dental fluorosis. However, some studies have shown an overlap in fluoride intake among caries and fluorosis cases which indicates that absolute fluoride intake may not be the best predictor of dental fluorosis. Several factors such as diet composition could alter the rate of fluoride bioavailability and, therefore, its absorption and consequent retention. This study aimed to assess fluoride bio-accessibility in a variety of foods and beverages most frequently consumed by young children.

Methods Ten commonly consumed baby food products from different UK and US manufacturers were purchased. Individual foods and mixed meals were prepared using low F water (LFW; 0.13mg F/L) or F water (FW; 0.9mg F/L) according to the manufacturer’s instructions and analysed in triplicates for F concentration by an acid-diffusion method. The bio-accessibility of the prepared samples was measured using a static in vitro digestion model.

Results Fluoride concentrations of the samples and percentages of fluoride bio-accessibility of the samples are presented in the table. The results show that the bio-accessibility of fluoride in baby foods is always < 100% and often limited, suggesting reduced absorption and retention. Further work is required to confirm this observation.

<table>
<thead>
<tr>
<th>Baby Foods</th>
<th>F concentration (µg/g)</th>
<th>Bio-accessibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit-based baby food</td>
<td>0.124</td>
<td>40</td>
</tr>
<tr>
<td>Vegetable-based baby food</td>
<td>0.238</td>
<td>24</td>
</tr>
<tr>
<td>Meat-based baby food</td>
<td>0.223</td>
<td>25</td>
</tr>
<tr>
<td>Baby cereals</td>
<td>0.169</td>
<td>22</td>
</tr>
<tr>
<td>Baby cereals and fruit-based baby food</td>
<td>0.203</td>
<td>31</td>
</tr>
<tr>
<td>Infant milk formula reconstituted with LFW</td>
<td>0.097</td>
<td>50</td>
</tr>
<tr>
<td>- FW</td>
<td>0.575</td>
<td>64</td>
</tr>
<tr>
<td>Baby cereals prepared with Infant milk formula</td>
<td>0.143</td>
<td>10</td>
</tr>
<tr>
<td>- ready-to-drink</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Infant milk formula reconstituted with LFW</td>
<td>0.249</td>
<td>10</td>
</tr>
<tr>
<td>- Infant milk formula reconstituted with FW</td>
<td>0.582</td>
<td>41</td>
</tr>
</tbody>
</table>
Evaluation of Partial Dental Restorations in a French Adult Population

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Objectives Based on a cross-sectional observational study on the evaluation of dental oral health (Resto Data) in a French adult population, the aim of the present study was to assess the quality of partial restorations according to their characteristics and location.

Methods The study included 76 investigators and 822 patients from dental hospitals and private dental offices (422 and 400, respectively). Partial restorations were evaluated using nine selected and simplified FDI criteria (3 to 8, 11, 12, and 14). The results were examined according to tooth location, number of restored surfaces, type of restoration, and filling material used.

Results Among the 4612 partial dental restorations evaluated, 91% were direct restorations mainly filled with composite-resin materials (61%). The distribution of all restorations was carried out according to the location (25% for anterior and 75% for posterior restorations) and the number of surfaces with a majority of 1- and 2-surfaces (43% and 33%, respectively). Globally, 44% of these fillings were considered as failed according to FDI criteria, requiring their repair or replacement. More precisely, the failure rate was generally related to one or even two associated criteria scored ‘clinically unsatisfactory/poor’ (n=1,489). The esthetic criteria ‘color match’ was the most frequent reason for failure (n=912). Overall, the most frequently encountered failure rates concern restorations with 3-surfaces or more (61%) and glass-ionomer cement restorations (76%) with no difference between anterior and posterior restorations.

Conclusions The study highlighted the type and quality of restorations observed in French adult patients from dental hospitals and private dental offices. The use of FDI criteria in assessing dental restorations would allow dental practitioners to take into consideration the favorable and unfavorable factors related to the durability of their dental care and repair or replacement needs.
**Improving Diagnosis and Management of Burning Mouth Syndrome**

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**Objectives** Complex approaches to diagnosing and managing patients presenting with chronic burning sensations on healthy oral mucosa did not bring us closer to treatment success superior to placebo. Our aim is to explore and utilize one particular property of BMS, that symptom decreases during meals. This property is mentioned in literature, but never reached the deserved attention. It is not universally accepted, nor it is included in any of existing definitions of BMS. Most clinicians would encounter it among patients, but there are no estimates of extent of this property in BMS population. The aim of this study is to determine this effect’s extent, as well as to find out whether it is primarily effect of mastication or gustatory stimulation.

**Methods** We report 49 consecutive BMS patients seen due to spontaneous oral burning, with normal oral mucosa at the sites of pain. We timed the onset of alleviation and resolution of pain following beginning of chewing a piece of paraffin or melting a candy. We also measured the time to symptom return, following the stopping of the respective stimuli. Symptom oscillations were recorded by 0-10 Numerical Pain Rating Scale.

**Results** In all but one subjects (N = 48) there was an improvement, 90% had a temporary complete remission to at least one of the stimuli. Improvement begins during the 1st minute, and maximum effect is obtained within 3 minutes. Wearing out of effect upon the end of the stimulation occurs within minutes, returning to symptom level before start of stimulation.

**Conclusions** Every but one consecutive patient felt marked improvement, most of them having complete response. This reliable property solves both major issues regarding BMS: its accurate and fast diagnosis (without any expenses) and temporary symptom management (without any side effects). This property deserves inclusion in definition of BMS.

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**Influence of Risk Factors on Caries Disease of Adult Population**

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**Objectives** Based on a clinical oral health assessment study (Resto Data) in a French adult population, the objective of the study was to assess the influence of different risk factors on caries disease according to age.

**Methods** In this study 76 investigators included 822 patients from hospital dental clinics and private practices (422 and 400, respectively) to be as representative as possible of the general population. Standardized clinical examinations were used to record DMF-T and caries lesions. In addition, a questionnaire was used to determine patient status, including data on lifestyle, diet, oral health behavior, local disorders, alcohol, tobacco, medications, and diseases.

**Results** The DMF-T index was 6, 11, and 15 for each age group 18 29 years, 30 59 years, and over 60 years, respectively. On average, 48% of patients had at least one caries (all ICDAS levels) and 38% had dentin caries (ICDAS 4,5,6) (33% office-based and 42% hospital-based). 10% of patients had 5 or more caries. Caries prevalence decreased with age.

85% of patients reported brushing at least twice daily nevertheless 34% had a plaque index ≥ 2.

In all groups, the most important risks were the presence of plaque, new patients, and irregular visits. In addition, for the youngest group, not having had orthodontic treatment increased the risk.

Sugary or energy drinks were also a significant risk factor, especially for patients under 60.

Brushing techniques are also very important. The use of electric toothbrushes was a protective factor in the elderly.

**Conclusions** The study highlights the relationship between patients’ risk factors and the presence of caries. It takes into account all the influencing factors and shows their impact according to the age of the patient.
**P238**

**Repair or Replace Failed Restorations? Systematic Review and Meta-Analysis**

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**Objectives** To conduct a systematic review to gather and compare survival data between repair and replacement techniques in failed restorations of primary and permanent teeth.

**Methods** The systematic searches were performed in PubMed/MEDLINE, Scopus, Web of Science, Embase, and OpenSigle databases. Two independent calibrated reviewers (kappa=0.87) assessed as inclusion criteria: (1) Repair or replacement studies, (2) success, longevity, or survival data, (3) randomized clinical controlled trials; and for the exclusion criteria (1) Loss to follow-up over 30%, (2) less than 12 months follow-up, (3) anterior teeth. The risk of bias was assessed by the RoB 2 tool, and the certainty of the evidence was assessed using the GRADE tool. Meta-analysis was conducted considering the treatments' survival rate.

**Results** We identified 4,070 potentially relevant publications, after removing duplicates and assessing title and abstracts, 2,115 studies were ineligible because they were not about repair and replacement, did not have percentage survival data, or were not RCTs. Seven remaining articles were assessed for the exclusion criteria, and we found all papers for full-text analysis. Three studies were included in the quantitative analysis, all evaluating permanent dentition. A standard follow-up time between studies was collected, and the pooled survival rate between studies was 99% after three years. There was no statistically significant difference between approaches and no heterogeneity between studies. All included studies had a high risk of bias. The certainty of the evidence for the outcome measure of success between repair and replacement interventions in failed restorations is very low.

**Conclusions** The longevity for both techniques appears to be similar. However, the certainty of the evidence is very low, and the trials have a high risk of bias.

**P239**

**Evaluation of the Electronic Faces Thermometer Scale (EFTS) in Children**

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**Objectives** Children’s possibility to communicate pain experiences is often challenged and need to be supported in the dental setting. A digital self-assessment tool, the electronic Faces Thermometer Scale (eFTS), has been developed and is currently evaluated in dental and medical settings in an international collaboration. Comparisons are made with the Color Analog Scale (CAS), a well-established and validated clinical pain scale. The dental evaluation is currently carried out in Sweden and this study evaluates the agreement between eFTS and CAS during pediatric dental treatment.

**Methods** The first 20 children in the evaluation (7 girls, 13 boys; aged 10-17 yrs) are included in this report. Depending on dental treatment needs they were divided into two groups no procedural pain (N=7) and procedural pain (N=13), respectively. Repeated pain measures were carried out in each individual using the two measures (eFTS and CAS) following a standardized protocol. To compare the agreement between eFTS and CAS intraclass correlations (ICC) were calculated.

**Results** There was a good agreement between pain measures using CAS and eFTS. ICC for all registrations was 0.98 (95% C.I. 0.96-0.98). Corresponding data for procedural pain group was 0.98 (95% C.I. 0.96-0.99), and for no pain group 0.95 (95% C.I. 0.88-0.98).

**Conclusions** This first report on agreement between eFTS and CAS shows promising results. This is important and encouraging as digital self-assessment can provide solutions for a better person-centred care in pain management and promote child participation in dental treatment.
**P240**

**Supernumerary Elements in Children With Microcephaly**

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**Objectives** To clinically analyze 50 children with microcephaly resulting from congenital Zika virus syndrome (CZVS), in order to observe the prevalence of supernumerary elements and compare with the same rates reported in the literature for normoreactive children.

**Methods** A sample of 50 children between 18 and 36 months of age, with microcephaly and part of a special child care association in Salvador, Bahia (Northeast Brazil), was clinically examined by two examiners, under natural light and using disposable retractors for better visualization of structures. This study was approved by the ethics committee.

**Results** As observed, two children (4% of the sample) had multiple supernumerary elements and one child (2% of the sample) had only one supernumerary element, totalling 6% of the sample with the alteration.

**Conclusions** Although investigations with larger samples are necessary, we were able to conclude that children with microcephaly have a higher incidence of dental number anomalies when compared to the population of normoreactive children at the same age range.

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**P241**

**Literacy on Oral Health of Parents of Children With SCZV**

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**Objectives** The purpose of this study is to associate parents' oral health literacy with the experience of dental caries in children with Congenital Zika Virus Syndrome.

**Methods** This study is a descriptive observational study was carried out, involving 30 pairs of parents and children, with the objective of associating parents' oral health literacy with the experience of caries disease in children with Congenital Zika Virus Syndrome. The ABEP socioeconomic questionnaire was used, a tool to assess the literacy level of parents with the BREALD-30 and a survey of children's ceod and CPOD.

**Results** The results showed that the literacy level (BREALD-30) related to social class B to C had a BREALD-30 average of 22.47 and classes D to E, a result of 19.93. Regarding the ceod/CPOD = 0, the BREALD-30 resulted in 21.24 and the ceod/CPOD > 0 with 21.11. Classes B to C had an average ceod/CPOD of 1.87 and classes D to E had an average ceod/CPOD of 1.07.

**Conclusions** The conclusion, there was no influence of caregivers' literacy with ceod/CPOD in children; ceod/CPOD and the caregivers' literacy was not related to social classes.

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**P242**

**Assessment of the Lingual Frenulum in Newborns**

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**Objectives** The objective of this study was to carry out the evaluation of the lingual frenulum in a newborn in the rooming-in of a State maternity hospital

**Methods** The lingual frenulum of 1846 normoactive newborns were evaluated in a state maternity hospital from 2019 to 2021 through the application of an evaluation protocol proposed by Martinelli 2013.

**Results** 165 (8.94%) had scores that indicated some alteration of the lingual frenulum, of these 123 (74.55%) had scores that indicated direct interference with breastfeeding and underwent frenotomy during hospital stay, 42 (25, 45 %) should return for reassessment because they presented scores indicative of doubtful and were rescheduled for a new evaluation in 30 days. 37 (88.1%) babies were reassessed and 5 (11.9) were absent, of the 37 assessed, 17 (45.95%) underwent frenotomy and 20 (54.05%) did not require surgical intervention.

**Conclusions** We conclude that the evaluation of the lingual frenulum allowed the diagnosis of ankyloglossia and the interference with breastfeeding in a hospital environment, which made possible a differential diagnosis for early frenotomy.
PER-IADR Oral Health Congress 2022 in Marseille

P243  
**Stainless Steel Crowns Versus Composites After Primary Molars Pulpotomies: 30-Months-Results**

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**Objectives** The aim of this clinical trial was to evaluate the 30-months survivals of pulpotomized primary molars restored occlusally with a resin composite (RC) as compared to stainless steel crown (SSC).

**Methods** 130 primary molars from 44 children aged between 5 and 9 years with at least one primary molar requiring pulpotomy treatment were selected. The primary molars were restored either with a SSC in case of proximal cavities or with a RC (GC Gradia-Direct Posterior, Japan) in case of occlusal cavities. An acceptance questionnaire was done orally immediately after the treatment and discomfort and/or pain of the patients were asked by phone on the following day. All restorations were evaluated after 1, 3, 6, 12, 24 and 30 months.

**Results** Initially 93 SSCs (69.4%) and 41 RCs (30.6%) were placed on primary molars, approvals of SSCs by patients were low but no discomfort was claimed the following day. At 12 months, 3 teeth were exfoliated, all restorations were intact, 17.4% of SSCs had tenderness to percussion and 12.5% of RCs had marginal discolorations. At 30 months, 65.6% of SSCs, 26.8% of RCs were exfoliated, for the remaining restorations, 6.3% of SSCs and 10% of RCs had marginal defects.

**Conclusions** Due to ethical reasons, we had to restore only occlusal cavities with RCs, the comparison of RC with SSC can be bias. Since the unesthetic appearance of SSC is the discontent of the child patient, within limits of this study, we may recommend applying RC to occlusal cavities for the endodontic treatment of the primary molars.

P244

**Oral Health Perceptions Related to Dietary Risk Factors in Early Childhood Caries**

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**Objectives** Early childhood caries (ECC) is a common chronic childhood disease with multifactorial etiology including poor parental feeding practice. Frequent intake of sugar-sweetened snacks and drinks are considered as a risk factor for ECC. Little is known about immigrant parents’ oral health related knowledge and attitudes being potential influences the early establishment of children dietary habits.

**Aim:** To assess knowledge, beliefs and feeding practice allied to ECC among parents with immigrant background living in Norway.

**Methods** A structured interview was performed with parents of non-western origin with newborn infants (0-6 months) to explore their knowledge and attitude towards infants’ feeding practice as well as their own sugar consumption.

**Results** Of those interviewed (345), 98% stated that they are willing to control their children’s sugar intake and 90% believed that it is easy to adopt routine for that. Among those 23%, believed that they might occasionally found it difficult to control their children’s sweets intake. Almost 30% of the respondents found that it is stressful to stand against their children demands for sweets and 10% perceived that giving their children sweets will help to improve children’s behavior. Notably, most of the study participants considered that frequent sugar intake leads to ECC. They did not believe, however that night frequent breastfeeding, for longer period, can be one of the priming causes of ECC. The participating parents reported daily (44.3%) and weekly (26.4%) sugar intake mostly in terms of sweetened tea and coffee.

**Conclusions** Parents with non-western origin presented with good knowledge related to children’s oral health. Some parents however, presented with unsatisfactory oral health related attitudes, which might negatively affect the corresponding feeding practice and dietary habits of their children. These findings highlight the importance of providing parents with an early dietary advice and guidance for prevention of ECC among their children.
Risk Factors for Repeated Pediatric Dental Treatments Under General Anesthesia
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Objectives The need for dental rehabilitations under general anesthesia (DRGAs) is continuously increasing, particularly for dental treatment of children. The null-hypothesis of this retrospective cohort study was that there were no differences between the demographic and anamnestic data, dental status, and performed treatments during DRGA between the children who received DRGA once and those who received at least two DRGAs in a private pediatric dental practice.

Methods Demographic and anamnestic data, dental status (DMFS/dmfs), and treatments (direct or indirect restorations, endodontic treatments, extractions, fissure-sealings) performed during DRGA were retrospectively analyzed from the electronic dental charts of 1155 children that received at least one DRGA between October 2016 and December 2021. Data were analyzed non-parametrically (Mann–Whitney U or χ² tests; α=0.05).

Results The median age of all children was 5 years at time of their first DRGA. The rate of repeated DRGAs was 9%. When comparing the 1051 patients who received one DRGA to those 104 who received at least two DRGAs, the latter group was significantly younger at their first DRGA (p=0.001), comprised significantly more patients with history of preterm birth (p=0.013) and reported significantly more often the current use of a baby bottle (p<0.001). There were significantly fewer treatments (regardless of type; p≤0.003) in the second DRGA than at the first except for extractions.

Conclusions Conclusions: This retrospective study showed that young age at first DRGA, history of preterm birth, and current use of baby bottle may be risk factors for repeated need of DRGA. For preventing repeated DRGAs, it might be worthwhile to consider a less conservative treatment approach in favor of extractions, particularly if children receive their first DRGA at a rather young age. Adherence to post-operative recall appointments seems crucial to avoid repeated need of DRGAs.

Schoolchildren’s Treatment Needs and Caries After 2-Years of Professional Toothbrushing
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Objectives This study aimed to compare the effect of professional toothbrushing (PTB) on the presence of dental caries and treatment needs of schoolchildren. Additionally, the caries prevalence and the treatment needs were compared according to two criteria: Nyvad caries detection system and World Health Organization (WHO).

Methods Children (4-9-year-old, n=263) from a public primary school in Petrópolis (Brazil) were clinically evaluated regarding dental caries and oral hygiene at baseline and every six months for two years. Children were randomly assigned to: PTB once a month (test) or PTB every six months (control). Chi-square-test, McNemar-test and binary logistic regression analyses were used (p<0.05).

Results A total of 6,232 tooth-surfaces in primary dentition, and 10,975 tooth-surfaces in permanent dentition were evaluated. When using both Nyvad and WHO criteria, most surfaces were scored as sound or arrested caries lesions in both dentitions. Treatment needs were low and there were no statistical differences between PTB groups (p>0.05). Furthermore, the caries prevalence using Nyvad criteria was higher compared to that of WHO criteria for both dentitions (p<0.05). For the primary dentition, the WHO criteria resulted in higher treatment needs compared to the Nyvad criteria (p=0.001), while for the permanent dentition the opposite was observed (p=0.001).

Conclusions No difference was found between PTB performed once a month or once every six months with regards to the presence of dental caries and treatment needs among Brazilian schoolchildren. In the primary dentition, the use of the Nyvad criteria resulted in higher caries prevalence, while the WHO criteria resulted in higher percentages of treatment needs. In the permanent dentition the use of the Nyvad criteria resulted in higher caries prevalence and higher percentages of treatment need compared to the use of the WHO criteria.
**P245**

**Oral Care for Inpatients With Dysphagia; Exploring Barriers and Facilitators**

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**Objectives** It is well established that high quality oral care is important in the overall care of patients who develop dysphagia post-stroke. This qualitative research project aims to explore the barriers and facilitators to carrying out an oral cavity assessment and providing oral care to inpatients with dysphagia on the acute care Stroke Unit of Cork University Hospital.

**Methods** Focus group methodology was used. Three focus groups were conducted in the Stroke Unit of Cork University Hospital with a total of seventeen participants. Each focus group consisted of a multidisciplinary group of health professionals currently working on the unit and caring for patients with dysphagia post-stroke. The focus groups were digitally recorded and professionally transcribed. Transcribed data were analysed by thematic analysis.

**Results** The first theme identified was the importance of oral care. Specifically, the participants related the importance of this to the prevention of systemic disease, maintenance of dignity and a step towards return to normal living for this group of patients. Themes related to the barriers to providing oral care for these patients included a lack of awareness of protocols and procedures, time pressures, lack of confidence and lack of specific training in oral care for patients with dysphagia. Themes related to the facilitation of oral care for this population included teamwork and availability of suction and oral care aids.

**Conclusions** It is clear that oral care is viewed as an important part of the overall care of inpatients with dysphagia post stroke. Multiple barriers exist however to the provision of high quality oral care for this patient group. Opportunities exist for multidisciplinary interventions to improve the oral cavity assessment and oral care provided on the Stroke Unit.

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**P246**

**Prosthetic Crowns Presence Increase Residual Teeth Number in the Elderly**

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**Objectives** The aim of this study was to measure the effect of the presence of Prosthetic Dental Crowns (PDR) on residual teeth of old people at the end of their lives.

**Methods** We have recruited in the geriatric day hospital (CHU Bordeaux France) a sample of 230 elderly people with a mean of 83,8 years, the mean of residual teeth was 17,8 and average of PDR present was 3,05. In this sample people retained at least one residual tooth. Our sample was divided in two groups of 115 people: In the first one (control group) people was without PDR. In the second one (experimental group) people have at least one PDR. We have measure in the two groups the number of residual teeth.

**Results** In experimental group the mean of PDR present was 6,11 and the mean of residual teeth was significantly higher (mean:21,27±7,88SD) than in control group (mean:14,33±9,04SD) (p< .000 Friedman ANOVA).

**Conclusions** It can be measured in elderly patients that the installation of PDR during their lives has allowed greater storage of residual teeth on the dental arch. These results validate the use of this treatment in this retrospective study.
P247
Stage-Model Intervention to Enhance Adherence in Obstructive Sleep Apnoea Patients
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Objectives The study aims to assess the effectiveness of a stage-matched intervention on adherence to mandibular advancement appliances (MAA) in participants with obstructive sleep apnoea (OSA).

Methods Fifty-six participants (Adults 18 years or over) with newly diagnosed OSA were enrolled in the study and randomised into intervention care (IC) and standardized care (SC) groups. Participants in the SC group received routine care whilst participants in the IC group received the stage-matched intervention, developed using the behaviour change model, the Health action process approach (HAPA). Data indicating MAA adherence was collected both objectively and subjectively, from micro-sensors embedded in the MAA design and sleep diaries, respectively at 3- and 6-months. In addition, a range of questionnaires was designed to assess risk perception, outcome expectancy, and self-efficacy (SEMSA) and quality of sleep (PSQI & ESS) and life (EQ-5DL), socio-economic and social support scales will be used.

Results The mean objective adherence for 30 participants at 3-month (IC = 15, SC = 15) was 2.02 vs 2.63 hours/night in the IC and SC group respectively. Whilst, the mean objective adherence for 25 participants at 6-month (IC = 10, SC = 15) was 2.42 vs 3.21 hours/night for IC and SC group respectively. No correlation was seen between ESS (p = 0.24), PSQI (p = 0.96), social support (p = 0.52), socio-economic position (p = 0.96) and mean adherence. However, linear regression for adherence at 3-months presented a positive coefficient for risk perception (p = 0.035) and outcome expectancy (p = 0.003).

Conclusions The interim analysis demonstrate the stage-matched intervention does not enhance adherence to MAA at 3-month follow-up. Notwithstanding this, adherence might be dependent on predictors such as risk perception and outcome expectancy.

P248
Sex as a Mediator of Malocclusion Perception and Impact on Quality of Life in Adolescents
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Objectives One third of the population has a clear need for orthodontic treatment, however, this estimate depends on the population and the perceived need for treatment of them. Malocclusion is considered a public health problem, as it has a high prevalence and can negatively affect quality of life. Consequently, the need for treatment emerges to restore the functional and psychosocial aspects of patients affected by this disorder. In this sense, the objective of this study was to assess if the adolescent's sex is a factor that interferes in the self-perception of malocclusion and in the impact on their quality of life.

Methods An observational cross-sectional study was carried out with 386 adolescents aged 11 to 14 years in public and private schools in the city of São Paulo, with cluster sampling, where children within schools and schools within the region were randomly selected. The severity of malocclusion was measured by the Orthodontic Treatment Need Index and the impact on oral health-related quality of life with the application of the Child Perceptions Questionnaire (CPQ11-14).

Results The results showed that boys had a significantly lower average quality of life when compared to girls, which means that, for males, the severity of malocclusion impacts less on quality of life when compared to females.

Conclusions Thus, the sex factor must be considered in decision making and indication of orthodontic treatment, since females have a greater negative impact on quality of life.
P250
Relationships Between Dental Calcification and Vertebral Maturation in Mp3cap Stage
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Objectives The aim of this study is to evaluate the relationships between maxillary canine, mandibular second premolar and mandibular second molar calcification stages and cervical vertebral maturation stages of patients in mp3cap stage detected by hand-wrist radiography.

Methods This descriptive study was designed as a cross-sectional research. Our study consisted of 30 patients who were randomly selected from the orthodontic clinic and who were in the mp3cap stage. Lateral cephalometric and panoramic radiographs taken simultaneously with hand-wrist radiographs of all patients were collected. The patients ranged in age from 10 years 4 months to 14 years 9 month. The calcification stages of maxillary canines, mandibular second premolars and mandibular second molars were evaluated using the Demirjian method.

Results In this study, the patients in the mp3cap stages in both gender groups were mostly found in the CS3 (46.6%) vertebral maturation period. According to chronological age, men enter the mp3cap stage approximately 1-2 years later than women. For this reason, more advanced stages have been observed in the maturation of the teeth compared to the Demirjian method. As a result of the this examination that used Demirjian method, it was found that maxillary left canines generally were at H stage; mandibular left second premolars were at F stage in females and at H stage in males; mandibular left second molars were at F stage in females and at G stage in males.

Conclusions In disadvantaged clinics that cannot reach cephalometric and hand-wrist radiography, it is possible to examine the calcifications of the teeth in panoramic radiographs with the help of the Demirjian method, which will give an idea about the bone development of the patient.

P251
Immunological Potential of HMSC Secretome is Dependent on Tissue Source
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Objectives Human mesenchymal stem cell (hMSC) secretome contains immunomodulatory, trophic, and protective factors, and have via paracrine action substantial potential in regenerative medicine. Capability to modulate immune responses is of particular interest for the treatment of chronic inflammatory diseases. The hMSCs isolated from different tissue sources are believed to have differences in secretion profiles of immunomodulatory cytokines.

Methods This study performed a comparative proteomic-based analysis of hMSC secretomes (n=3 per tissue source) derived from bone marrow (hBMSC), periodontal ligament (hPDLSC) and dental pulp (hDPSC). The hMSC secretomes were obtained under xenofree conditions and analyzed by liquid chromatography tandem mass spectrometry (LC/MS-MS).

Results Approximately 3400 proteins were identified within each hMSC secretome tissue group. Number of unique proteins for each origin were: 4 for hBMSC, 4 for hPDLSC, and 9 for hDPSC. Hierarchical clustering displayed the highest degree of similarity between hDPSC and hPDLSC. A total of 1212 proteins were differentially secreted between the three groups, reaching statistical significance after one-way ANOVA testing (permutation-based FDR ≤ 0.05). Gene set enrichment analysis revealed differences of Hallmark gene sets between the three groups. Proteins corresponding to the Hallmark Inflammatory Response gene set were up-regulated for hPDLSC compared with hBMSC and statistically significant up-regulated compared with hDPSC. Hallmark Complement gene set - protein components of the innate immune response, were statistically significant up-regulated for hPDLSC compared with hDPSC and hBMSC.

Conclusions These results suggest that hMSC secrete almost identical proteins independently of origin. Nevertheless, the levels of secreted proteins differ significantly between all tissue groups. Bioinformatic analysis elevates hPDLSC secretome as the most potent hMSC source for immunomodulation. This may have implication on the selection of hMSC for future clinical studies.
Screening SHED Gene Expression in Xeno-Free Medium by Next-Generation Sequencing

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Objectives Stem cells from human exfoliated deciduous teeth (SHED) are dental stem cells which are easy to access by noninvasive procedures. Due to the advantages of SHED in self-renewal and differentiation into various cell types, SHED became an important candidate for tissue regeneration. Using fetal bovine serum in culturing cells for therapeutic application in human presents some limitations including risk of disease transmission from microorganism contamination and immunogenicity from xenogenic proteins. The aim of this study was to evaluate the effect of xeno-free medium on SHED gene expression.

Methods SHED were divided into 2 groups according to culture medium, which were xeno-free medium (XFM) and serum-containing medium (SCM) group. SHED were cultured until 80-90% confluence, then the cells in each group were extracted for RNA and analyzed for gene expression by next-generation sequencing (NGS). Genes with significantly differential expression more than 16 folds were selected and studied for their function by Gene ontology (http://geneontology.org/) and Uniprot (https://www.uniprot.org/) resources.

Results The results from NGS demonstrated 261 significantly differentially expressed genes between 2 groups. There were 24 upregulated genes and 25 downregulated genes in SHED cultured in XFM. There were 18 genes expressed only in SHED cultured in XFM group and 13 genes expressed only in SHED cultured in SCM group. Two of 18 genes expressed only in XFM were amphiregulin (AREGB) and vanin1 (VNN1), which function in inhibition of host immune response. On the other hands, IL-6 is a gene expressed only in SCM, which is a well-known pro-inflammatory cytokine.

Conclusions The results illustrated a trend that XFM could decrease immune response of SHED, which may be useful for future cell therapy applications. However, further studies are required to verify the effect of xeno-free medium on cell proliferation, differentiation and immune responses both in vitro and in vivo.
3D Printed PLATMC Scaffolds Induce Consistent Host Immune Response

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Objectives Significant effort has been devoted to fabrication techniques used to design scaffold with suitable properties for bone regeneration. Highly porous and degradable scaffolds are conventionally fabricated from Poly(L-lactide-co-trimethylene carbonate) (PLATMC) using salt-leaching methods. 3D-printing technology enables control over the mechanical properties and porosity of the material, which enhances the regenerative potential of the scaffold by improving cell attachment and distribution.

The aim of the present experimental in vivo study was to investigate the host response to 3D-printed PLATMC scaffolds in comparison to salt-leaching scaffolds.

Methods PLATMC scaffolds were enrolled in a subcutaneous model in Lewis rats and harvested at 4 days and 8 weeks. Twenty-three relevant chemokines were investigated using Bioplex protein assay (BIORAD). Histological analysis was performed on formalin-fixed paraffin-embedded sections. Immunohistochemical (IHC) staining for macrophages (CD68) was quantified as percent positive pixels using Color-Deconvolution Algorithm (Aperio).

Distribution of chemokine data in 3D-printed and salt-leached scaffolds were investigated by principal component analysis. Linear regression models were constructed to compare chemokine and macrophage levels according to scaffold fabrication method. Data analysis was conducted in R v4.2.0 with 0.05 set as the significance limit.

Results 3D-printed group showed less variation in chemokine expression levels than the salt leaching group. However, three chemokines were significantly downregulated: KC/Gro (neutrophil activating protein, p=0.018), Interferon gamma (INF-γ) inducing factor (natural killer cell chemoattractant, p=0.014), and IFN-γ (macrophage activating factor, p=0.03). Similarly, Histological examination revealed less infiltration by mononuclear inflammatory and multinucleated foreign-body giant (MNFG) cells and lower CD68 signal in the 3D-printed group (p<0.001).

Conclusions 3D-printing of scaffolds offered more consistent host immune response in comparison to salt-leaching. However, the resulting response is characterized by low infiltration of immune cells. Further investigations are needed to improve the immunogenicity of 3D-printed PLATMC scaffolds.
Expression of Hippo Pathway Components in Adult Dental Mesenchymal Stem Cells

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Objectives The aim of this research is to discover if there is an expression of the Hippo pathway as regulation mechanism for tissue growth and cell proliferation. Hippo pathways are responsible for the regulation of cell proliferation, but unlike other signaling pathways, it involves a ligand-receptor pairing, regulated by biochemical, physical and cellular activities. YAP and TAZ are transcriptional coregulators that do not contain DNA-binding domains. The primary binding partner of YAP/TAZ are TEAD. Once activated, the Hippo pathway limits tissue growth and cell proliferation by phosphorylating and inhibiting YAP/TAZ. In contrast, when the Hippo pathway is off, YAP/TAZ are dephosphorylated and translocated into the nucleus, where they bind to TEAD to induce transcriptional programs important for cell proliferation, survival, and migration. Specifically the Hippo pathway regulation is not static, but rather dynamic. That is why YAP/TAZ is under and rapid phosphorylation and dephosphorylation although much less is known about the regulation of the responsible phosphatase.

Methods Stem cells from apical papilla (SCAP) and stem cells for adult dental pulp (DPSC) were cultures and tryptosphinized (0.05 % trypsin-EDTA, Gibco) (5 min, 37 °C). After that cDNA was isolated with FastLane Cell cDNA kit (50), Cat. No: 215011, which directly isolates cDNA from cultured cells without RNA purification. Then the cDNA is transferred to RT² Prolifer PCR Array (384-Well [4x96] Format) for Human Hippo Signaling, Cat.No 330231 PAHS-172ZA and a PCR on qRTPCR was administered.

Results We defined the expression of ACTG1, AMOTL, CASP3, CRB,LAT ,YWHA, TEAD, ACTB, B2M, etc. We characterized a panel of Hippo related genes in groups such as: Upstream Hippo Signaling Regulators, Hippo Core Kinase Complex, Downstream Mediators of Hippo Signaling, Hippo Signaling Target Genes, Contact Inhibition, Cell Polarity, Scaffolding Proteins.

Conclusions DPST and SCAP are two types of stem cells with a very specific regulation and cell growth. The proof of expression of this markers send us one step closer to understanding their regulation of expression and the proliferation and differentiation potential in the adult organism.
Definition of Different Cell Populations in Heterogeneous Primary DFSC Cultures
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Objectives The goal of the research is to characterize quantitatively the different subsets of cells in a mesenchymal DFSC culture through High content imaging analysis. We speculate in the primary cellular cultures isolated from native tissues which have similar fibroblast-like morphology, different subsets of cells exist, and these different subsets are providing the multilineage potential of the said cultures.

Methods Dental follicle of retained third molars were collected after routine extractions in Dental faculty (Medical university – Sofia) after informed consent was obtained from the patients. Pericoronal DFSC were seeded in 96 well plates and subdued to High-Content Cellular Analysis. The IN Cell Analyzer 6000 imaging system (GE Healthcare, Pittsburgh, USA) enabled identification and quantification of the cell markers expression. A total of 36 fields of view per well were photographed at x20 magnification. Cells were divided according to cell/background fluorescence intensity values measured by In Cell Analyzer work station 3.7.3 (x64) software (GE Healthcare): positive expression – fluorescence intensity >10; no expression/autofluorescence – fluorescence intensity <10.

Results We revealed that all selected markers are strongly expressed by approximately one third of the cell population. Therefore, nearly one third of the isolated cells are non-differentiated stem cells based on the amount of cells expressing stem cell markers. Of all the stem cell markers in the present study, CD90 was strongly expressed by the highest number of cells (49%). The expression of the following markers was found to be statistically significant when compared to control: CD90, CD117, vimentin, ALP, Sox9, CK10, p63, FGF7(KGF), Col3. The cells from each of the fractions may co-express markers from more than one group, as the cell culture is heterogeneous.

Conclusions Primary DFSC cultures contain different subsets of cells, and these different subsets are providing the multilineage potential of the said cultures.
in Vitro Three-Dimensional Organotypic Culture Model of the Oral Mucosa

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Objectives The main objective of the Oral-Muco-Chip (OMC) project is the development of a suitable microenvironment for oral epithelial, endothelial, and minor salivary glands stem cells (mSG-SC), which will recapitulate in vitro the normal oral mucosa physiology. Two-dimensional (2D) in vitro models have been utilized for decades to elucidate the nature of adverse cytotoxic effects of dental biomaterials on various types of oral cells. Nevertheless, limiting factors in terms of direct in vitro to in vivo extrapolation (IVIVE) of the 2D results limit their prognostic value. Three-dimensional (3D) systems seem more appropriate since they provide higher levels of cellular differentiation and tissue organization closer resembling the in-situ situation.

Methods In the first approach (Static), oral epithelial cells will be seeded in Air Liquid Interface (ALI) on the upper chamber for 15 days and then human gingival fibroblast (HGF) will be encapsulated with HUVEC in a 3D hydrogel in transwell plates. For the second approach (dynamic), we will seed oral epithelial cells in the microfluidic platform in the same way as described for the static culture that delivers both type of media in a similar manner like the flow rates of the in vivo model as shown in Figure 1.

Results We have established a static transwell organotypic co-culture of oral epithelial with gingival fibroblast and endothelial cells using region specific matrix, closely mimicking the native oral mucosa region.

Conclusions This model will lead to translational applications for studying and developing personalized medicine protocols and provide an essential tool of risk assessment procedures in Dentistry in compliance with the European Commission (EU) policies for Replacement, Reduction, Refinement and Rehabilitation (the 4 Rs’ rule) of animal use in regulatory testing.

Figure 1. Mimicking in vivo microenvironment architecture. A) static transwell culture of oral mucosa, B) dynamic culture using a microfluidic system
**P257**

**Novel Fluorescent RNA Binding dye in Adult Mesenchymal Stem Cell.**

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**Objectives** The aim of this research is to test the cytotoxicity of new developed florescent dyes. The dye has a specific connecting properties to RNA. The nucleoid acids binding dye is checked for specificities for RNA and/or DNA. The difference of the structure, charge and the hydrophilicity is expected to influence cellular permeabilisation and cellular toxicity.

**Methods** A 3 step protocol was followed in the assessment of the novel dye. Mesenchymal stem cells from apical papilla were isolated. When reaching 80% confluence, they were trypsinized (0.05 % trypsin-EDTA, Gibco) (5 min, 37 °C). Further on the cells are transfened into two different vessels. Half of the cells are seeded in 48 well plate and the other half in 25 cm² plastic flask. The dye is administered in the 48 well plate and the cell is immediately placed for observation in InCell Analyzer 6000 (GE HealthCare). In the flasks the florescent dye is added 24 hours prior the flow-cytometry Cellular penetration was observed and recorded on Incell Analyzer 6000 (GE HealthCare) for a period on every 30 minutes for 6 hours. The differentiation between the apoptotic and alive cells was made by Annexin V kit for flow-cytometry on Navios (BioRad).

**Results** For High through-put florescent cellular analysis, the apoptotic cells were visualized with FITC-conjugated anti-annexine V antibody. The nuclei were counterstained with DAPI. The results show that the tested dye has an insignificant cytotoxic effect on the cells. It shows a specific binding with RNA components, especially in the nucleus. With the High through-put florescent cellular analysis the cells shows a significant signal from the nucleus of a living cell, not in an apoptosis state.

**Conclusions** New specific dyes are urgently needed for understanding cellular processes. Only one RNA specific binding dye is available on the market. Development of new nontoxic dyes discriminating between RNA and DNA or binding specific regions in the proteins will give us valuable tools for understanding the transmembrane transport, RNA translation and protein folding and interactions.

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**P258**

**Dental Pulp Stem Cells: a Promising CMT1A in Vitro Model.**

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**Objectives** Charcot-Marie-Tooth disease type 1A (CMT1A) is the most common demyelinating peripheral neuropathy, caused by a duplication of the peripheral myelin protein 22 (PMP22) gene. It is mainly expressed by Schwann cells, where it plays an essential role in developing, maintaining, and stabilizing healthy myelin. The use of autologous primary human Schwann cells is restricted, and animal models are often of limited translational value. Therefore, there is no cure for CMT1A. We aim to develop a human dental pulp stem cell (DPSC)-based in vitro model to study CMT1A-related disease mechanisms. DPSC are isolated from the dental pulp of third molars and can be classified as a subtype of mesenchymal stem cells (MSC).

**Methods** Multiple healthy donor-derived DPSC lines were differentiated towards Schwann cells (DPSC-SC), and donor-based variability was determined through qPCR and immunocytochemistry (ICC). Next, we used lentiviral vectors to induce PMP22 overexpression in the DPSC-SC to mimic CMT1A.

**Results** After DPSC differentiation, Schwann cell markers P75NTR, S100B, SOX10, Laminin211, and Laminin411 showed stable and similar expression in DPSC-SC from different donor lines. Furthermore, following lentiviral transduction, mRNA levels confirmed successful PMP22 overexpression in the DPSC (150%, p=0.1609). By diluting the lentiviral vector titer to the extent that only 10% of the cells were transduced, we obtained DPSC and DPSC-SC that overexpress only one extra copy of the PMP22 gene, mimicking the PMP22 duplication in CMT1A.

**Conclusions** In conclusion, our data confirm a high consistency in our Schwann cell differentiation protocol. Additionally, we successfully induced PMP22 overexpression in both DPSC and DPSC-SC. Alternative approaches for PMP22 overexpression, such as CRISPR/Cas9, may provide an alternative approach. Further research should focus on validating this novel human in vitro CMT1A model.
M2 Muscarinic Receptor Modulates Dental Pulp Stem Cell Behaviour
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Objectives Acetylcholine (ACh) has been found to be expressed by non-neuronal cells and act as an important cyto-transmitter involved in tissue homeostasis. ACh signalling is mediated by the nicotinic (nAChR) and muscarinic (mAChR) families of receptors. mAChRs are metabotropic receptors which signal via G protein mediated pathways and modulate cellular phenotype and function. Mesenchymal stem cells (MSCs) express mAChRs and their influence on regenerative potential has been partly investigated. Dental Pulp Stem Cells (DPSCs) are multipotent cells that reside in the post-natal dental pulp and share similar characteristics with MSCs. However, the presence of functional mAChRs, associated signalling pathways and their role in modulating the function of DPSCs has yet to be explored.

Methods Expression of mAChRs was investigated in human DPSCs and functionality was explored using subtype specific mAChR agonists and antagonists. DPSCs proliferation, migration, and differentiation were evaluated in response to mAChR activation. Additionally, relevant downstream mAChR signaling pathways were explored.

Results DPSCs predominantly express the M2 mAChR. Furthermore, a selective M2 mAChR agonist inhibited DPSCs proliferation and migration; without affecting cell viability. Indeed, upon activation of the M2 mAChR, DPSC maintained their stemness properties and could resume proliferation once the agonist was withdrawn. Furthermore, M2 activation hindered osteogenic differentiation of DPSCs, resulting in reduced mineral deposits. Expression of key osteogenic genes in response to osteogenic differentiation media was found to be inhibited by the presence of the M2 mAChR agonist. Furthermore, the data suggest that M2 mAChR signaling is mediated by the downstream extracellular signal-regulated kinase 1/2 (ERK1/2) pathway as the M2 mAChR agonist induced the phosphorylation of ERK1/2 and upregulated expression of the MAPK1 and proliferating cell nuclear antigen (PCNA) genes.

Conclusions The data indicates that the M2 mAChR is expressed by DPSCs and plays an important role in modulating their regenerative potential.

Characterisation of Periodontal Stem Cells Isolated From Osteoporotic Patients
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Objectives Osteoporosis and its medications have possible implications on patients' periodontal condition and healing. Little is known about the regenerative capacity of osteoporotic periodontal ligament stem cells (OP-PDLSCs). Therefore, the aim of the project is to characterise PDLCs isolated from postmenopausal osteoporotic patients and compare them to those isolated from healthy individuals. The objectives include the assessment of their ability to form colonies, long-term growth characteristics and their osteogenic differentiation potential in vitro.

Methods PDLCs were isolated from healthy and osteoporotic patients. The primary characterisation of PDLCs was attained by: colony forming unit-fibroblast assay (CFU) after 14 days in culture, and population doubling time (PDT) assay, followed up for 2 months for each cell type. For osteogenic differentiation assessment, passage 6 cells from both groups were seeded and cultured in osteogenic media (basal media +100µM L-ascorbic acid and 10µM dexamethasone) for 2, 3 and 4 weeks (basal media used as control). Cultures were assessed using Alkaline Phosphatase Staining (ALP) assay and Alizarin Red Staining (ARS) and quantitative assay.

Results The primary characterisation results of CFU indicated an average of 64±23 colonies for healthy PDLCs and an average of 44±23 colonies for OP-PDLCs. The average for PDT for all passages was 2.07±0.5 days for healthy PDLCs and 1.66±0.12 days for OP-PDLCs. For both the control and osteoporotic donors, ALP staining was more intense under osteogenic conditions. ARS preliminary results indicated that mineralisation was the highest at week 3 in healthy PDLCs grown in osteogenic conditions (0.025mM±0.004) whereas it remained unchanged in OP-PDLCs (average 0.0135mM±0.002).

Conclusions The results suggest that there is a trend of lower clonogenicity, and mineralisation capacity of OP-PDLCs compared to healthy controls which can affect their regenerative capacity to heal the periodontium.
P294
Heterogeneity of Multipotent Cleft lip and Palate-Derived Fibroblasts
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**Objectives** Fibroblasts isolated from cleft lip and palate (CLP) tissue possess multipotent characteristics, including osteogenic potential. Hence, they may represent an intriguing cell source for alveolar bone regeneration in cleft-affected patients. However, before testing fibroblasts’ in vivo potential, a complete and thorough characterization of their nature and origin is warranted.

**Methods - Results** Using qPCR analyses and consulting public databases, we conceived the heterogenous origin of CLP fibroblasts. Lip is a complex structure composed of skin and oral mucosa, with related fibroblasts. Both skin and oral mucosa fibroblasts can be clustered into papillary, reticular, pro-inflammatory and mesenchymal fibroblasts. Mesenchymal fibroblasts are believed to be the fibroblasts possessing differentiative capacity. Using CLP lip biopsies, we were able to separate skin from oral mucosa as well as the papillary fibroblasts from the reticular ones. This allowed us to comparatively study the multipotent character of all the individual fibroblast groups, which revealed that all these populations possess similar osteogenic and colony-forming units efficiency. These observations let us speculate the co-localization of mesenchymal fibroblast with the secretory papillary and reticular clusters. To select and enrich for them we performed a single-cell clonal analysis, gaining some clones displaying increased levels of the mesenchymal fibroblasts-associated markers (POSTN and ASPN). However, these clones did not possess superior multipotent capacity compared to the parental CLP strain.

**Conclusions** Our results clearly indicate the heterogenous nature of CLP fibroblast cultures. We shows that no single subpopulation possessed superior potential when compared to the parental CLP strain, even when we enrich for the mesenchymal fibroblast cluster. There is therefore no need for fibroblast cluster selection, as each single fibroblast can contribute to the success of the whole population. Indeed, even fibroblasts not showing multipotent traits might be beneficial, as they contribute to the cell numbers, thereby reaching the critical cellular mass required for potential regenerative therapies.

P262
Deterioration of Dental Restorative Materials Under Erosive and Mechanical Wear
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**Objectives** Dental restorative materials are subject to various kinds of wear such as erosion, attrition, and abrasion. Prior to choosing an appropriate material for a patient, particularly for use in the posterior, load-bearing areas, it is crucial to have knowledge about the physical and chemical properties of the materials. The objective was to evaluate the impact of erosion combined with abrasion and attrition on the mechanical and colour properties of a composite-resin material (Filtek Z250®) and two glass ionomer cements (Fuji IX GP® and Equia Forte®) by evaluating surface roughness, hardness and colour stability.

**Methods** The specimens (n=6) of each materials group (n=3) were prepared in metal molds, grinded, polished and left in artificial saliva (37°C, 24h). Samples were subjected to abrasive (brushing simulator), erosive (liquid cycler) and attrition (chewing machine) tests. Each test was mimicking six months of wear exposure. Baseline and final measurements of surface roughness, hardness and colour shade were recorded and compared. Data were analysed using GraphPad Prism 9.3.1. Statistical significance was set at p<0.05.

**Results** A significant increase in surface roughness was observed in all three materials after the experiment, with the largest roughness difference found for Filtek Z250®. Hardness was significantly reduced in Fuji IX® while Filtek Z250® and Equia Forte® had a similar change in hardness values. Regarding color stability, Filtek 250® and Fuji IX® became one shade darker and lighter, respectively. Equia Forte® became greyish after the experiment (Table 1).

**Conclusions** Even though Equia Forte® showed superior results compared to Fuji IX®, its performances are rather limited in comparison to Filtek Z250®. Therefore, composite resin should be given priority when used for posterior, load bearing restorations.
Table 1. Mechanical properties and colour shade changes in three dental materials following wear exposure (mean values ±SD; n=6).

<table>
<thead>
<tr>
<th></th>
<th>Filtek Z250® (n=6)</th>
<th>Equia Forte® (n=6)</th>
<th>Fuji IX® (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Final</td>
<td>Baseline</td>
</tr>
<tr>
<td>Roughness (Ra, µm)</td>
<td>0.13 (±0.01)</td>
<td>0.92 (±0.09)*</td>
<td>0.67 (±0.08)</td>
</tr>
<tr>
<td>Hardness (VH 0.5 kg, MPa)</td>
<td>113.6 (±2.95)</td>
<td>100.7 (±1.00)</td>
<td>72.2 (±1.63)</td>
</tr>
<tr>
<td>Colour** (Vitapan®)</td>
<td>A3</td>
<td>A4</td>
<td>A3</td>
</tr>
</tbody>
</table>

*Significantly different from baseline, (p < 0.05). **A2, A3, A4 – “reddish brownish”; C2 – “grayish”.

P263

Optical Characteristics of Short Fiber-Reinforced Composite in Bilayered Structure

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Objectives The aim of this in vitro study was to optically evaluate required thickness of particulate filled composite (PFC) used on top of short fiber reinforced composite (SFRC) in direct bilayered composite structure.

Methods Three groups of SFRC materials were studied: everX Flow Dentin (eXD), everX Flow Bulk (eXB) and everX Posterior (eXP) (GC, Tokyo, Japan). Samples were made in 4mm deep cavities in transparent molds with SFRC substructure and a PFC veneer (GC G-ænial Universal Injectable A3). Six different SFRC-PFC-ratio combinations were tested with each SFRC material to find out the required PFC thickness where the human eye cannot detect the underlying SFRC material compared to PFC reference cylinder. The used PFC thicknesses were 2.0, 1.5, 1.2, 1.0, 0.7 and 0.5mm. Test samples (n=5/thickness/material) were made in two steps and colours were optically measured with a spectrophotometer (CM-700d, Konica-Minolta, Tokyo, Japan).

Results The results are presented in the Table. In all SFRC materials color change values (ΔE) acted almost inversely proportionally to PFC thickness (R² > 0.85). All three tested SFRC materials had a statistically significant difference in ΔE-values, when veneering PFC thickness was 1.0mm (p< 0.05). In the groups where PFC material thickness was 2.0mm, the flowable SFRCs (eXD and eXB) showed statistically significant difference compared to packable SFRC (eXP) (p< 0.05).

Conclusions The thickness of veneering PFC affects the optical properties of bilayered restorations. The thickness of PFC should be taken into consideration, when using bilayered SFRC structures in highly aesthetic areas.

Effect of PFC thickness on color change(ΔE) / SFRC-material

<table>
<thead>
<tr>
<th>PFC thickness(mm)</th>
<th>eXD</th>
<th>eXB</th>
<th>eXP</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFRC-material</td>
<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>2.77 (0.06) c</td>
<td>3.53 (0.08) a</td>
<td>3.16 (0.12) b</td>
</tr>
<tr>
<td></td>
<td>1.51 (0.14) l</td>
<td>1.78 (0.15) i, e</td>
<td>1.86 (0.10) j</td>
</tr>
<tr>
<td></td>
<td>1.17 (0.14) k</td>
<td>1.22 (0.07) f</td>
<td>1.56 (0.07) n, f</td>
</tr>
</tbody>
</table>

Levels not connected by same letter are significantly different.
P264
To Ascertain the Current Level of Understanding Regarding the use of Light Curing Units Among General Dental Practitioners in Ireland
Elizabeth A. Ryan
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Objectives To gather information from general dental practitioners in Ireland on their knowledge on the practical use and technical information of their light curing units (LCUs), as well as LCU safety awareness.

Methods A hard copy of the questionnaire was posted to 700 dentists registered with the Irish dental council. The survey consisted of 14 questions in total, relating to participants demographics, and to the use, monitoring and replacement of their LCUs. Responses were analysed using IBM® SPSS Statistics (Version 27).

Results Eighty-seven dentists (12.4%) returned the completed questionnaire.

The average time for curing a 2mm increment of resin composite was 22 seconds. Regarding curing times, 68% of respondents said they would follow the restorative materials manufacturers’ guidelines. The remaining 29% stated they either follow the manufacturer of the LCU or their clinics’ guidelines for curing times.

When irradiance values were concerned, 57% of dentists stated they have LCUs with irradiance values >2000, mW/cm², and 25% have units with irradiances of between 1000-1499mW/cm². 12% of respondents were not sure of the output of their LCUs.

In response to when light curing units would be replaced one third of dentists (number) stated they would be replaced their LCUs when they break. 50% said they would replace their LCU on average every 3-4 years, 12% rarely or didn’t know how often they replaced their units. 72% never check the output of their LCU.

In terms of eye care, 24% of the dentists stated they only occasionally or never use blue filtering orange shields. Only 10% of dentists said they would air-cool the tooth between supplemental light exposures.

Conclusions This study highlights a wide variation in knowledge among general dental practitioners in Ireland regarding LCUs technical data and their usage. Larger studies and qualitative data collection may help better understand this variation.

P265
Influence of Ionizing Radiation on Restorative Materials With Fluoride Release Effect
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Objectives Radiotherapy plays a key role in the treatment of the early and advanced stages of head and neck cancer. Although marked by a series of acute and late side effects that affect the cells of numerous anatomical structures in the area of the oral cavity, head and neck, it also shows an impact on restorative materials. To date, there is still no consensus on the effects of radiotherapy on the mechanical properties of fluoride-releasing materials.

Methods In the research, we used samples of materials with the effect of releasing fluoride and composites: Equia Forte HT (GC, Japan), Cention N (Ivoclar, Liechtenstein), Tetric EvoCeram (Ivoclar, Liechtenstein) and Tetric Power Fill (Ivoclar, Liechtenstein). The examined parameters were mass, surface roughness, microhardness and color change.

Results Cention N and Tetric PowerFill showed stability in the mass, while the surface roughness did not change in any of the examined groups. Resistance to microhardness change was shown by Cention N, Tetric PowerFill and Tetric EvoCeram, color change was significant in all groups. The brightness did not change in one group, and resistance to chromaticity changes was demonstrated by Cention N and Tetric EvoCeram.

Conclusions The obtained results suggest that Cention N can serve as choice material in patients who are undergoing head and neck radiotherapy due to the mechanical stability of the material and the depot effect of fluoride release. However, further research is needed.
High Wettability of Paste-Type Alginate Impression Material for Precise Impression
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Objectives A new impression material we have released in Japan, Aroma Injection (ARI), is a paste-type alginate impression material filled in a cartridge. This product can be used as wash material for combined impression with conventional alginate impression materials. ARI has enough hardness and consistency to take precise impression. Since alginate materials are water-based, ARI is compatible with wet tooth surfaces unlike non-water-based materials such as polyether and silicone. Wettability is the key to success in making accurate impressions and prevent re-impression in the presence of exudate and saliva on surfaces of teeth. The aim of this study is to evaluate the wettability of ARI and compare it with other impression materials, agar, polyether, and silicone.

Methods ARI (GC, Japan, alginate), Aroma Loid (ARL: GC, Japan, agar), Impregum Penta Soft (IPS: 3M ESPE, USA, polyether), and Take1 Advanced (TOA: Kerr, USA, silicone) were used for measurement of contact angle and dynamic wettability. For the contact angle, each material was mixed, flattened on a glass slide, wetted with a droplet of distilled water 1min after the mixing (2.0µL), measured at 10s, and statically analyzed (Tukey, p<0.01). For the dynamic wettability, each material was mixed, attached to the test plate, and tested 1min after the mixing for 20s (test speed: 0.5mm/min, test depth: 1mm, immersion into water).

Results The contact angle of ARI was significantly lower than polyether and silicone while there were no significant differences between ARI and agar. The result of dynamic wettability test corresponded to the result from the contact angle test.

Conclusions ARI has much higher wettability than polyether and silicone impression materials. The result from this study indicates ARI can take precise impressions even if there is any exudate and saliva on surfaces of teeth and prevent clinical failure from occurring.

Fig. Contact angle of impression materials
Tukey-Kramer test (p<0.01)
*Same superscript indicates no statistically difference.
Effect of Reinforcement on Selected Properties of Resin-Modified Glass Ionomer Cements

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Objectives Minimal intervention and biomimetic approach are growing trends in modern dentistry aiming to restore tissue natural way and preserve healthy tissue. Those requirements are matched by resin-modified glass ionomer cements (RMGICs). However, RMGICs suffer from weak mechanical properties, thus, reinforcement of RMGIC restorative materials is essential. This study investigated the reinforcing effect of discontinuous-glass fiber fillers on fracture resistance of RMGIC. In addition, the effects on depth of cure were also detected.

Methods Experimental fiber-reinforced RMGIC (Exp-RMGIC) was prepared by adding (20 wt.%) discontinuous-glass fiber of around 100 µm in length to the powder of RMGIC. The fracture resistance FR (V-notch method) and depth of cure DC (ISO 4049 method) were determined for experimental and control materials (Fuji II LC, GC; Surefil One, Dentsply). The FR measurements were carried out using hand mixing (Manual) and mechanical (Capsule) mixing. Scanning electron microscopy was used to evaluate the microstructure of the materials.

Results Fiber-reinforced RMGIC had significantly higher fracture resistance (p<0.05) but slightly lower depth of cure compared to control materials. SEM analysis showed random orientation and protruded (pullout) fiber ends at fracture surfaces of Exp-RMGIC matrices.

Conclusions The use of discontinuous-glass fiber fillers with RMGIC matrix is novel reinforcement and yielded superior toughening and flexural performance compared to conventional resin-modified glass ionomer like materials.

<table>
<thead>
<tr>
<th></th>
<th>Exp-RMGIC-Manual</th>
<th>Exp-RMGIC-Capsule</th>
<th>Fuji II LC-Manual</th>
<th>Fuji II LC-Capsule</th>
<th>Surefil One-Capsule</th>
</tr>
</thead>
<tbody>
<tr>
<td>FR (N/mm²)</td>
<td>16 ± 4</td>
<td>18 ± 3</td>
<td>11 ± 3</td>
<td>12 ± 2</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>DC (mm)</td>
<td>--</td>
<td>2.3 ± 0.1</td>
<td>--</td>
<td>3.0 ± 0.1</td>
<td>3.2 ± 0.2</td>
</tr>
</tbody>
</table>
Discoloration Resistance Property of Light-Cured Characterization Materials
Kimitaka Tanazawa, Shuji Kariya, Futoshi Fujisima
Research & Development Dept., GC Corporation, Kasugai-shi, Japan

Objectives Light-cured characterization materials like OPTIGLAZE color (GC) are used to staining or glazing on wide range of resin materials such as CAD/CAM hybrid resin blocks, composite resins and denture teeth. However, there is a risk of discoloration due to a large amount of resin components in such products, resulting in the aesthetics get poor. In this study, it was evaluated the discoloration resistance property of light-cured characterization materials in several environments.

Methods OPTIGLAZE color Clear (GC, OC), MAZIC Glaze Clear (VERICOM, MG) and VITA AKZENT LC GLAZE (VITA, VA) were examined. They were applied and light-cured with Labolight Duo (GC) on 1mm thickness of CERASMART270 A2 LT which was sandblasted with 50µm alumina and treated with G-Multi PRIMER (GC) in advance, in accordance with manufacturer’s instructions. The specimens of each group were immersed in distilled water, coffee or red wine at 37 degree C for 1 week, respectively. The color of each specimen, before and after the immersion, were measured with spectrophotometer (SD 7000, NIPPON DENSHOKU), and ΔE*ab was calculated (n=3). All results were analyzed by Tukey-Kramer test (p<0.05).

Results The color change in distilled water immersion was apparently smaller than in coffee and red wine. Both in coffee and red wine immersion group, OC showed significantly lower ΔE*ab (3.48 and 9.66, respectively) than that of MG (6.12, 11.39) and VA (7.58, 16.75). Furthermore, those difference could be clearly seen visually. These results were considered to be largely influenced by the difference in the structure and amount of the monomers used.

Conclusions It was seen that the light-cured characterization materials tend to be discolored under strong color components like coffee and/or red wine. However, OC was apparently less discolored compared to the others and could be expected to be kept the aesthetics for long lasting clinical cases.

Fig. Results of discoloration resistance test.
Resin Composites Surface Hardness and Roughness Evaluation in Drink Solutions

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Objectives The aim of this study was to investigate the effects of artificial saliva and drink solutions on surface roughness and hardness of 2 different resin composites: Herculite XRV (HE) and Harmonize (HA) (Kerr) after six months of aging.

Methods Fifty-four composite specimens were prepared in an oxygen-free environment and polymerized. Six specimens were immediately investigated (T0), while forty-eight specimens were soaked into different drink solutions (artificial saliva, cola, ethanol), sealed into PET bottles and incubated at 37°C. Control specimens were kept in air. Specimens evaluation was made at T0 (n=6) and after 6 months (T3)(n=48). Twenty-four samples (12=HE,12=HA) were analyzed with an optical microscope equipped with a Confovis-structured-light camera used as profilometer. This system digitally elaborates a known size grid projected on the surface to be analyzed in order to evaluate the differential interference contrast. Twenty-four samples (HE=12, HA=12) underwent instrumented (depth-sensing) indentation test to evaluate hardness at room temperature.

Results A generalized linear mixed effect model has been applied considering composites, drink solutions and time as fixed effects. At T3, roughness appeared significantly increased in samples soaked in ethanol, compared with those in air, saliva and coke (respectively p=0.001,0.02 and 0.008). No statistical differences were observed between composites (p=0.071). Concerning surface hardness, values at T3 for both HA and HE soaked in air, saliva and coke were significantly higher than in ethanol (p=0.001 except for coke where p=0.038).

Conclusions HE and HA significantly increased their roughness after 6 months of immersion in ethanol, with no significant differences between composites. Both HA and HE showed a significant hardness decrease after 6 months in ethanol.

Dexamethasone-Doped Polymeric Nanoparticles Induced Dentin Remineralization

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Objectives To analyze the effect of doped polymeric nanoparticles (NPs) with zinc and dexamethasone in dentin remineralization.

Methods Polymeric NPs of 250 nm in diameter were synthetized and after doped with dexamethasone (Dex) or Zinc (Zn) by immersion in a saturated aqueous solution. Sound dentin discs (1 mm in thickness) were acquired from human molars with informed consent from donors (1906/CEIH/2020). Specimens were metallographically polished and etched with 35% phosphoric acid. An ethanol suspension of NPs, Dex-NPs or Dex+Zn-NPs was applied on dentin surfaces. A control group without NPs was also included. Nanomechanical properties (i.e. complex modulus) were assessed using a Hysitron Ti Premier nanoindenter, after 24 h and 21 d of immersion in phosphate buffered saline solution. ANOVA, Student Newman Keuls and Student t tests were performed (p<0.05). After nano-DMA specimens were fixed in a solution of 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer for 24 h and observed by scanning electron microscopy (SEM) to ascertain changes in dentin morphology.

Results After 24 h storage, the dentin specimens that were not covered with NPs attained the highest complex modulus in GPa (control=69.35), and the rest of the groups follow the trend: NPs (19.10)>Dex-NPs=8.86. After 21 d, complex modulus mean values were as follow: control (91.14)<NPs (113.92)=Dex+Zn-NPs (156.37)<Dex-NPs (234.82). Clear signs of dentin remineralization were observed after SEM analysis of Dex-NPs and Dex+Zn-NPs treated dentin surfaces.

Conclusions After 21d Dex+Zn-NPs and Dex-NPs produced dentin remineralization. An improved in nanohardness, complex modulus and tubules closure were observed in both groups. This work was funded by the Ministry of Economy and Competitiveness and European Regional Development Fund (PID2020-114694RB-I00 MINECO/AEI/FEDER/UE). M.T.-O. is fellow FPU of the Ministry of Universities [FPU20/00450].
P271
Mechanical Stability and Ion Release of Experimental Fiber-Reinforced Bioactive Composite.
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1Stick Tech GC, Kaarina, Finland, 2Turku Clinical Biomaterial Center, Turku, Finland, 3Dental school, University of Turku, Turku, Finland, 4Stick Tech Ltd., Turku, Finland

Objectives This study aimed to investigate the ions release and mechanical stability of a novel bioactive fiber-reinforced flowable resin composite (Bio-SFRC) in comparison with different commercial ion-releasing materials.

Methods Flexural strength (n=8) of experimental Bio-SFRC and other ion-releasing materials (Biodentine, TheraCal LC, Fuji II LC and Surefil One) was assessed before and after hydrothermal accelerated aging. Ion concentrations of silica and phosphate were measured after 1, 2, 3, 4, 7, 10, 14, 21 days of specimen immersion in simulated body fluids (SBF) using UV-vis spectrometry. SEM and EDS were used to evaluate the microstructure on the top surface of the materials after immersion. Data were statistically analyzed with analysis of variance ANOVA (p = 0.05).

Results Bio-SFRC showed higher flexural strength before (134.9 MPa) and after (63.1 MPa) hydrothermal aging compared to commercial tested materials (p<0.05). Flexural strength significantly decreased after aging (p<0.05) except for Fuji II LC which showed no significant differences. Ion release data showed that experimental Bio-SFRC slowly released phosphorus ions. However, Biodentine and TheraCal LC seemed to have the strongest ability to form CaP, which can be seen as decreasing phosphorous concentration and as precipitation on material surface. On the other hand, only Biodentine and Theracal LC were found to release silicon. Surefil One and Fuji II LC seemed to be more stable materials.

Conclusions The advantages of fibrous structure and slow release of ions suggest that experimental Bio-SFRC is promising bioactive material to promote mineralization of surrounding tissues while keeping the integrity of restoration.

P272
Functionalizing Resin Composites With Bioactive Glass: the Effect on Microhardness
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Objectives To evaluate the effect of functionalizing experimental composites with two types of bioactive glass on their microhardness.

Methods Eight experimental composites were prepared by adding variable amounts of two bioactive glass types (conventional 45S5 formulation and a customized fluoride-containing bioactive glass) as functional fillers to experimental light-curable Bis-GMA/TEGDMA-based composites. A commercial composite was used as a reference, while the experimental composite containing only inert glass was used as a negative control. Light-cured specimens were aged for 1 day or 30 days in distilled water at 37 °C and Vickers microhardness was measured under a load of 100 g and a dwell time of 15 s. Eight specimens were used per experimental group, whereas five separate indentations were performed on different spots of the same sample and averaged to account for heterogeneity. The data were statistically analyzed using three-way ANOVA with the following factors: bioactive glass type, bioactive glass amount, and aging time (overall α=0.05).

Results Microhardness values (VHN) of experimental composites ranged from 47.4–68.7 (1 day) and 46.8–64.0 (30 days). A statistically significant effect of bioactive glass type and amount was identified, with the customized fluoride-containing bioactive glass leading to statistically similar or significantly better microhardness compared to the 45S5 composition. All of the composites functionalized with the fluoride-containing bioactive glass performed similarly or better than the negative control material. For most of the materials, the 30-day artificial aging exerted no effect on microhardness, while a significant microhardness reduction due to artificial aging was identified for the composites with 10 wt% of the 45S5 composition and 40 wt% of the fluoride-containing bioactive glass.

Conclusions Under the conditions of this short-term in vitro study, functionalizing experimental composites with a customized fluoride-containing bioactive glass led to improved surface microhardness compared to the traditional 45S5 composition.
Effect of Printer Types on Mechanical Properties of 3D-Printed Splints

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Objectives To evaluate the effect of 3D printer types on the mechanical properties of hard occlusal splints. Flexural strength, flexural modulus, and fracture toughness were evaluated.

Methods 40 bar-shaped specimens (KeySplint Hard, keyprint) were 3D printed and stored in water at 100°C for 16 hours, half of them were printed by LCD printer with 405 nm (Creo™ C5, PLANMECA) (Creo group), while the other half were printed by DLP printer with 385 nm (Asiga MAX™, Scheu-Dental) (Asiga group). Each group was divided into two subgroups based on the post-curing method: stroboscopic post-curing with and without nitrogen gas (n=10/subgroup). Flexural strength and modulus were evaluated. Additional specimens (n=32) were prepared and similarly divided for fracture toughness evaluation. Data were statistically analyzed with ANOVA and Tukey post-hoc analysis (α=0.05).

Results ANOVA showed that 3D printer type and post-curing method significantly affected the investigated mechanical properties as shown in Table (P<0.001). Stroboscopic post-curing with nitrogen gas caused a significant increase in flexural strength and modulus of specimens only in Creo group (P<0.001). Creo specimens post-cured with nitrogen gas had the significantly highest flexural strength and modulus when compared to Creo specimens post-cured without nitrogen, Asiga specimens post-cured with nitrogen and without nitrogen (P<0.001). Only in specimens post-cured with nitrogen gas, the flexural modulus and strength of Creo specimens were significantly higher than those of Asiga specimens (P<0.001). Meanwhile, the fracture toughness of Asiga specimens was significantly higher than that of Creo specimens (P<0.001), regardless of the post-curing method.

Conclusions 3D printer type and post-curing method can affect the mechanical properties of the investigated splint material. Using Creo™ C5 and stroboscopic post-curing with nitrogen gas can enhance the flexural strength and modulus, while using Asiga MAX™ can enhance the fracture toughness.

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Flexural strength (MPa) Mean ± SD</th>
<th>Flexural modulus (GPa) Mean ± SD</th>
<th>Fracture toughness (MPa m¹/₂) Mean ± SD</th>
<th>Broken ratio (%) of specimens within 12mm deflection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creo</td>
<td>SS</td>
<td>66.3 ±3.2ª</td>
<td>1.7 ±0.1ª</td>
<td>0.71 ±0.06ª</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>SS +N₂</td>
<td>80.5 ±1.6ª</td>
<td>2.1 ±0.1ª</td>
<td>0.69 ±0.05ª</td>
<td>40</td>
</tr>
<tr>
<td>Asiga</td>
<td>SS</td>
<td>73.6 ±1.8ª</td>
<td>1.8 ±0.1²ª</td>
<td>0.85 ±0.03ª</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>SS +N₂</td>
<td>73.8 ±1.5ª</td>
<td>1.9 ±0.1ª</td>
<td>0.83 ±0.03ª</td>
<td>100</td>
</tr>
</tbody>
</table>

Creo: Creo™ C5; Asiga: Asiga MAX™; SS: stroboscope, N₂: nitrogen gas. Same superscripted letters indicate groups not statistically significantly different when compared by Tukey multiple comparisons post hoc analysis (P>0.05).
Heat Generation of Two-Component Composites During Polymerization

Martina Hauner-Westphal, Johannes Schädlich, Andrea Cavalleri

Ivoclar, Schaan, Liechtenstein

Objectives
Investigation of the heat of polymerization of three two-component core-build-up-materials.

Methods
The following materials were tested:
Multicore Flow (MCF, Ivoclar)
Experimental Core-Build-Up-Material (ECM, Ivoclar)
Rebilda (REB, Voco)

A class II cavity was prepared in PMMA-based artificial teeth and equipped with a type-K-thermocouple. The material under investigation was placed in the cavity and after equilibration to 23°C, exposure to a dental-light-curing-unit (BluePhase-G4, Ivoclar-Vivadent) at 1200mW/cm² was conducted for 10s and 20s. The light-guide was placed in immediate material-contact and the temperature was recorded for 150s from starting light-exposure using a data logger. All experiments were duplicated (test-run-1&2).

Results
At 20s exposure, temperatures were in a narrow range of 53.4°C to 58.7°C for all tested materials. Notable temperature-differences between test-run-1 and/or test-run-2 were detected for ECM (5.6K/5.3K) and MCF (8.2K/0.6K), while REB was more uniform (0.7K/1.4K). ECM showed lower T_max than MCF. REB generated lower T_max than MCF and ECM but showed continued temperature-rise after end of light-exposure while MCF and ECM reached T_max during light-exposure. At 10s exposure REB was not completely cured and remained stuck to the light guide.

Conclusions
At 20s light-exposure, T_max was within the limits of accuracy in the same range for all products tested. All materials showed a lower temperature-rise after 10s-polymerization than after 20s. Deviations between test-runs are attributed to unavoidable small differences in material-volumes employed, thus reflecting clinical settings. At 10s exposure REB was not fully cured, in accordance with manufacturer’s recommended exposure-time of 20s.

Maximum temperature measured (Tmax)

<table>
<thead>
<tr>
<th>Material</th>
<th>Exposure [s]</th>
<th>Exposure time [°C]</th>
<th>test-run-1 [°C]</th>
<th>test-run-2 [°C]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECM</td>
<td>10</td>
<td>51.5</td>
<td>45.9</td>
<td></td>
</tr>
<tr>
<td>MCF</td>
<td>10</td>
<td>60.7</td>
<td>52.5</td>
<td></td>
</tr>
<tr>
<td>REB</td>
<td>10</td>
<td>42.3</td>
<td>43.0</td>
<td></td>
</tr>
<tr>
<td>ECM</td>
<td>20</td>
<td>53.4</td>
<td>58.7</td>
<td></td>
</tr>
<tr>
<td>MCF</td>
<td>20</td>
<td>56.9</td>
<td>57.5</td>
<td></td>
</tr>
<tr>
<td>REB</td>
<td>20</td>
<td>53.9</td>
<td>55.3</td>
<td></td>
</tr>
</tbody>
</table>
**P275**  
**Color Stability of Different CAD/CAM Blocks Against Coloration With Coffee**  
Pervin Uluğ, Elçim Çoban, Ayse Canan Tutku Çelik, Hayriye Esra ÜLKER  
selçuk university faculty of dentistry, Konya, Turkey  

**Objectives**  
This study evaluated color differences ($\Delta E_s$) of various computer-aided design/computer-aided manufacturing (CAD/CAM) blocks after immersion in coffee.  

**Methods**  
Specimens from different CAD/CAM materials [(Lava ultimate (resin nanoceramic), Ceresmart (Hybrid ceramic), Grandi (Nanohybrid composite)] were prepared in 10x10x2 mm dimensions and polished according to the manufacturer’s instructions (n=10 per series). The samples were kept in coffee in an incubator at 37 °C for 5 days for coloring. The CIE L*a*b* values of the specimens were measured using a spectrophotometer (Vita Easy Shade, Germany) on a white background before and after immersion coffee. The color change value, Delta E ($\Delta E$), was calculated. Data were analyzed by one-way ANOVA and post hoc Tukey's test. P value of less than 0.05 was considered statistically significant for all tests. In the measurement of coloration change, it was obtained by evaluating $\Delta E$ value over 3.3.  

**Results**  
The average $\Delta E$ values of the coffee-colored CAD/CAM samples were 5.06 in the Lava group, 3.18 in the Cerasmart group and 4.13 in the Grandi group. There was a statistical difference between the $\Delta E$ values of the coffee colored Lava group and the Cerasmart group (p <0.05). The coffee-colored Grandi group showed similar $\Delta E$ values with the other groups (p >0.05). The mean delta E values of Lava and Grandi groups were higher than the 3.3 value, which is perceived by the eye.  

**Conclusions**  
After 5 days of coloring with coffee, Lava and Grandi groups showed unacceptable color changes. Cerasmart group showed acceptable color change.  

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**P276**  
**Surface Gloss and Wear of Short-Fiber Reinforced CAD/CAM Composite Block**  
Sufyan Garoushi1, Eija Säilynoja2, 3, Roosa Prinsii, Bart Dopheide1, Pekka Vallittu1, Lippo Lassila3  
1GC Europe N.V., Leuven, Belgium, 2Stick Tech Ltd., Turku, Finland, 3Turku Clinical Biomaterial Center, University of Turku, Turku, Finland  

**Objectives**  
The purpose of this in vitro study was to determine the effects of different polishing protocols and tooth brushing on the surface gloss (SG) of an experimental short microfiber-reinforced CAD/CAM composite block (SFRC) in comparison with commercial CAD/CAM composite (Cerasmart 270, GC), as well as to evaluate the surface wear of the materials.  

**Methods**  
A total of 40 block-shaped specimens (14 mm length × 12 mm width × 2 mm thick) were prepared (n=20/material). Each group was subdivided into three subgroups (n=5), according to polishing protocol: Laboratory-machine polishing with 4000 silicon-carbide paper grit (G1). Chairside-hand polishing using a series of Sof-Lex spiral (G2). Laboratory-hand polishing (G3) using diamond impregnated spiral polishing system (EVE Diacomp Plus Twist) and a polishing paste (Gradia diapolisher, GC). Last subgroup was subjected to tooth brushing cycles for 30 min after grit paper polishing (G4). Glossmeter was used to determine the SG at 60° incidence angle. A wear-test of laboratory polished (4000 grit paper) specimens was performed using a chewing-simulator with 15000 cycles and wear depth (n=6) was assessed by 3D noncontact optical-profilometer. Scanning electron microscopy (SEM) analysis was performed. Data were analyzed using ANOVA (p = 0.05).  

**Results**  
Differences in SG were found according to the type of polishing protocol (p<0.05). No statistically significant differences was found between the materials after laboratory-machine or hand polishing (p>0.05). After 30 min of continuous tooth brushing cycles, SG values decreased significantly regardless of the material used. The lowest wear depth measurement was located for Cerasmart 270 (21 µm) which was not significantly different (p>0.05) from SFRC CAD (23 µm).  

**Conclusions**  
Incorporation of short microfibers to the composite of the CAD/CAM block did not negatively influence the surface gloss and wear of composite. The overall wear and gloss results obtained are in line with those reported in literature being clinically acceptable.  

<table>
<thead>
<tr>
<th>Material</th>
<th>SG (4000 grit)</th>
<th>SG (Sof-Lex)</th>
<th>SG (Laboratory-hand)</th>
<th>SG (30 min brushing)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFRC CAD</td>
<td>81.0</td>
<td>73.3</td>
<td>86.7</td>
<td>54.4</td>
</tr>
<tr>
<td>Cerasmart 270</td>
<td>83.4</td>
<td>88.8</td>
<td>88.8</td>
<td>56.6</td>
</tr>
</tbody>
</table>
Effect of Nitrogen Gas on Mechanical Properties of 3D-Printed Splint
Kanae Wada1, 2, Junichiro Wada2, 1, Mona Gibreel2, Tsutomu Iwamoto1, Noriyuki Wakabayashi3, Pekka Vallittu4, 5, Lippo Lassila2
1Department of Pediatric Dentistry / Special Needs Dentistry, Tokyo Medical and Dental University (TMDU), Tokyo, Japan, 2Turku Clinical Biomaterials Centre-TCBC, University of Turku, Turku, Finland, 3Advanced Prosthodontics, Tokyo Medical and Dental University (TMDU), Tokyo, Japan, 4Biomaterials Science Department, University of Turku, Turku, Finland, 5Welfare Division, City of Turku, Turku, Finland

Objectives To evaluate the effect of nitrogen gas during post-curing and water storage on the mechanical properties of 3D printed hard occlusal splints. The evaluated properties were flexural strength, flexural modulus, and fracture toughness.

Methods 40 bar-shaped specimens (KeySplint Hard, keyprint) were 3D printed (Creo™ C5, PLANMECA), half of them were dry-stored (dry group), while the other half were stored in water at 100°C for 16 hours (water group). Each group was divided into two subgroups based on the post-curing method: post-curing with stroboscope and nitrogen gas; or post-curing with only stroboscope (n = 10/subgroup). Flexural strength and flexural modulus were evaluated. Additional specimens (n=32) were prepared and similarly divided for evaluation of fracture toughness. Data were collected and statistically analyzed with ANOVA and Tukey post-hoc analysis (α = 0.05).

Results ANOVA showed that the post-curing method and water storage significantly affected the investigated mechanical properties as shown in Table (P<0.001). Water storage caused a significant reduction in the flexural strength and modulus of specimens post-cured without nitrogen gas (P<0.001). Water-stored specimens post-cured without nitrogen gas had the lowest significant flexural strength and modulus when compared to dry specimens post-cured without nitrogen, dry specimens post-cured with nitrogen, and water-stored specimens post-cured with nitrogen (P<0.001). Only in water-stored specimens, there was a significant difference in flexural modulus and strength between specimens post-cured with and without nitrogen (P<0.001). On the other hand, the fracture toughness of water-stored specimens was significantly lower than the dry-stored ones (P<0.001), regardless of the post-curing method.

Conclusions Water storage and post-curing method can affect the mechanical properties of the investigated 3D printed splint material. Stroboscopic post-curing with nitrogen gas can enhance the mechanical properties of 3D printed hard splints.

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Flexural strength (MPa) Mean ± SD</th>
<th>Flexural modulus (GPa) Mean ± SD</th>
<th>Fracture toughness (MPa m²) Mean ± SD</th>
<th>Broken ratio (%) of specimens within 12mm deflection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry group</td>
<td>SS</td>
<td>92.5 ± 3.1</td>
<td>2.2 ± 0.1</td>
<td>2.22 ± 0.15</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>SS + N₂</td>
<td>90.6 ± 4.0</td>
<td>2.1 ± 0.1</td>
<td>2.23 ± 0.17</td>
<td>60</td>
</tr>
<tr>
<td>Wet group</td>
<td>SS</td>
<td>66.3 ± 3.2</td>
<td>1.7 ± 0.1</td>
<td>0.74 ± 0.10</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>SS + N₂</td>
<td>80.5 ± 1.6</td>
<td>2.4 ± 0.1</td>
<td>0.69 ± 0.05</td>
<td>40</td>
</tr>
</tbody>
</table>

SS: stroboscope; N₂: nitrogen gas. Same superscripted letters indicate groups not statistically significant when compared by Tukey multiple comparisons post hoc analysis (P>0.05).
Mechanical Properties of 3D-Printed Provisional Material Compared to Milled Material

Vanessa Derendorf, Astrit Kastrati, Waltraut Loh, Annika Herr

Research and Development, Kulzer GmbH, Wehrheim, Germany, Kulzer GmbH, Hanau, Germany

Objectives

This investigation compares the mechanical properties of a new photopolymer with a milled provisional crown-and-bridge material by measuring flexural strength (FS), elastic modulus (EM), water sorption (WS) and solubility (SO).

Methods

Tested provisional materials were dima Print Teeth & Temp (dPTT; Kulzer GmbH), Telio CAD Disc (TD) and Telio CAD Block (TB; both Ivoclar Vivadent GmbH). Bar shaped specimens (n=5/material) were produced for FS testing and stored in water (24h, 37°C) before testing. 3-point bending was performed according ISO 10477 with a universal testing device (Zwick/Roell 2010) at a cross-head speed of 1 mm/min to determine FS. From the same measurement EM was calculated as secant (1N to 7N).

For WS and SO testing, round specimens (n=5/material) were produced from dPTT and TD. Specimens were conditioned in a desiccator (22h, 37°C), placed in a second desiccator (2h, 23°C), weighed and procedure repeated until a constant mass was achieved. Specimens were weighed again after water storage (7 days, 37°C), and after reconditioning. Testing and calculation according to ISO 10477.

Statistics were done using one-way ANOVA (LSD α = 0.05).

Results

See Table 1: Results.

The results of FS, EM and WS were significantly different between the materials. All results of FS, WS and SO fulfil the ISO 10477 requirements. For EM no ISO requirement is stated.

Conclusions

Within the limitations of this study, it can be stated that the new photopolymer dima Print Teeth & Temp exhibits good mechanical properties comparable to those of a milled reference product.

Table 1: Results

<table>
<thead>
<tr>
<th>Mean values (SD)</th>
<th>dima Print Teeth &amp; Temp</th>
<th>Telio CAD Disc</th>
<th>Telio CAD Block</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexural strength [MPa]</td>
<td>113.8 (2.3)</td>
<td>122.7 (2.3)</td>
<td>103.5 (5.8)</td>
</tr>
<tr>
<td>E-modulus [MPa]</td>
<td>3268 (49)</td>
<td>3031 (52)</td>
<td>2538 (87)</td>
</tr>
<tr>
<td>Water sorption [µg/mm³]</td>
<td>17.8 (0.3)</td>
<td>23.5 (0.4)</td>
<td>/</td>
</tr>
<tr>
<td>Solubility [µg/mm³]</td>
<td>0.1 (0.2)</td>
<td>0.4 (0.4)</td>
<td>/</td>
</tr>
</tbody>
</table>

Effect of Ultrasound on Setting Reaction and Microhardness of GIC.

Deena ALModdahi, Bandar Alshehri, Saroash Shahidi, Natalia Karpukhina

Queen Mary university of London, London, United Kingdom, Queen Mary University of London, London, United Kingdom

Objectives

To evaluate the effect of using radiant heat emitted by curing light and ultrasonic energy on the setting reaction using FTIR, 27Al MAS-NMR and mechanical properties of GICs using Vickers microhardness test.

Methods

Three conventional GICs, Fuji IX, Riva and Fuji Triage were studied. Ultrasonic excitation with a scaler tip for 55s or light-curing for 120s were used to accelerate the setting reaction and a control group of each material was left to set without activation. The samples were analysed using ATR-FTIR and 27Al MAS-NMR to monitor the cement setting reaction. The surface hardness of the tested GICs was determined using the Vickers microhardness test. The surface was subjected to 10 indentations and measured 1h, and 24h following material mixing. The data were statistically analysed by using one-way ANOVA and Bonferroni post-hoc tests.

Results

The FTIR and 27Al MAS-NMR spectra of the setting reaction of all tested GICs showed similarities after the ultrasound and heat application compared to the spectra of the self-cure cement samples. These external acceleration methods significantly increased the surface microhardness for all tested materials at an early setting time when compared to the same material which was left to be chemically set.

Conclusions

The use of an external energy source such as a light-curing unit or ultrasonic excitation though made no significant difference in chemical acceleration of the setting reaction, enhanced the mechanical properties of GIC during the early setting time.
Effects of Home-Bleaching Agents on Physical Properties of Dental Materials

Nazmiye Donmez1, Gökce Donmez Kiran2

1Restorative Dentistry, Bezmialem Vakif University, Istanbul, Fatih, Turkey, 2Specialist in Oral Health Center in Istanbul, Turkey

Objectives To evaluate the effects of two home bleaching agents on different restorative materials regarding surface roughness (SR) and color changes (CC).

Methods Specimens were prepared from ormocer-based nanohybrid composite resin (NCR) (Admira Fusion, Voco, Germany), and TCD-urethane matrix-based NCR (Charisma Topaz, Kulzer, Japan), high-viscosity glass ionomer cement (Equia Forte, GC, Japan), and glass carbomer (GC) filling material (GlassFill, GCP, Netherlands). One hundred twenty samples were prepared, polished, and subjected to thermal aging. A spectrophotometer (Vita EasyShade Advance 4.0, Germany) for color measurement, a profilometer (MarSurf M, Germany) for SR were used. Then the samples were randomly divided into three subgroups (n=10): Control (no bleaching), 10% Carbamide peroxide (CP) (Opalescence PF 10%), and 45% CP (Opalescence QuickPF 45%). After bleaching procedures (BP), all measurements were repeated and statistically analyzed (one-way ANOVA, Dunnet, Bonferroni tests and Student t-tests, p<0.05).

Results It was found that BP did not affect SR and the color stability of AF. When all groups treated with 10% CP were compared with each other, it was found that GC showed more CC than the other groups in terms of total CC, while the total CC of CT decreased. It was observed that SR of GC was different from other groups and had the highest SR. When all groups treated with 45% CP were compared with each other, it was observed that there was no statistically significant difference between them in terms of total CC (p>0.05). When evaluated in terms of SR, it was found that GC showed a significant difference from all other groups. It was observed that 10% CP application to EF increased total CC value, while it did not change in SR. It was found that 45% CP did not change SR and color values of EF.

Conclusions Old restorations with ormocer-based NCR may not need to be replaced when both 10% and 45% CP home-bleaching agents are applied. Since the other materials’ at least one parameter was negatively affected by BP, replacing the existing restorations after home BP can be advised.

Inlay Retained 3D Printed Three-Unit Fiber-Reinforced Composite (FRC) Fixed Dental Prostheses (FDP)

Jasmina Bijelic-Donova1, 4, Sufyan Garoushi2, 4, Milla Lähdenpera3, Lippo Lassila2, 4

1Department of Prosthetic Dentistry, Institute of Dentistry, University of Turku, Turku, Finland, 2Biomaterials Science, Institute of Dentistry, University of Turku, Turku, Finland, 3Faculty of Health and Well-being, Turku University of Applied Sciences, Turku, Finland, 4Turku Clinical Biomaterials Centre - TCBC, Institute of Dentistry, University of Turku, Turku, Finland

Objectives To evaluate the load-bearing capacities of 3D-printed inlay-retained three-unit fiber-reinforced composite (FRC) fixed dental prostheses (FDPs) with different designs.

Methods Forty FDPs were fabricated on metallic testing models. Five groups (n=8) were designed. In Group 1, FRC-FDPs were fabricated conventionally with fiber framework (everStick C&B) and resin composite (Gradia Plus). In Group 2, unreinforced FDPs were 3D-printed (GC Temp Print Light). In Groups 3, FDPs were first 3D-printed and space for the fiber framework was made by drilling. In Group 4, fiber frameworks were made and then scanned. This enabled designing and 3D-printing the FDPs with a groove on the gingival side for the fiber framework. In Group 5, the FDPs were designed and 3D-printed with a tunnel in the middle of the pontic for inserting the fiber bundle. 3Shape was used for designing and Asiga MAX for printing the FDPs. All FDPs were cemented with resin cement (Relay Ultimate) then loaded statically until fracture.

Results Conventionally fabricated FRC-FDP (Group 1) showed the greatest endurance (1150±262 N), which statistically was not different to Group 4 (1114±182 N) (p>0.05), but was significantly different to Group 2 (710±135 N), Group 3 (764±120 N) and Group 5 (652±152 N) (p<0.05). The difference among last ones was not statistically significant (p>0.05).

Conclusions The performance of fiber-reinforced 3D-printed prostheses was improved by fiber inclusion, but was highly dependent on the framework design. Drilling a space for the fiber framework caused microcracks, which weakened the material; whereas tunnel preparation hampered the placement of the fiber bundle in the designed place. Additional transversal fiber was not possible with the 3D-printing technique. Consequently, it could be concluded that this FRC-FDP fabrication technique requires future investigations. In this study, conventional handmade technique appeared the most reliable in terms of technique sensitivity and endurance.
**New Criteria for Standardized Evaluation of Sculptable Composite Handling**

Carola Pentelescu, Arnd Peschke, Ronny Watzke, Lukas Enggist, Ming Hu, Tatiana Glebova  
*RCC, IvoclarVivadent AG, Schaan, Liechtenstein*

**Objectives** The aim was to develop a standardized method to evaluate the handling properties of sculptable composites and to assess its usability and reproducibility.

**Methods** Five groups of dentists A-E (*table1*) were asked to evaluate 10 handling properties of a sculptable composite (TetricPrime, IvoclarVivadent AG, Schaan, Liechtenstein) according to a rating scale with 1=very good, 2=good, 3=satisfactory/poor, 4=unacceptable. Each handling property with rating scale is described in *image1*. Group A tested the material twice in a 12-month interval in order to assess the reproducibility of the evaluation method. Group B-E tested the material once to assess the usability of the investigation setup. Each dentist was asked to place a class I filling and a direct veneer on model teeth. Afterwards the rating for each handling property was recorded. Statistical analysis was performed by Mann-Whitney and Kruskal-Wallis test (*p*<0.05).

**Results** For group A there was no statistically significant difference between the means for overall handling at the first evaluation 1.45 (+/- 0.46) and at 12 month 1.43 (+/- 0.29). The results of group A=1.45 (+/- 0.46), B=1.44 (+/- 0.22), C=1.54 (+/- 0.39), D=1.35 (+/- 0.11), E=1.43 (+/- 0.26) were comparable and showed no statistical significance.

**Conclusions** All evaluator groups rated TetricPrime as excellent regarding the overall handling score (*classification defined in table 2*). The results showed that the users of group A generated similar results in two separate test-sessions at 12-month interval, so that the test method can be considered time-reproducible with a slight intragroup variation. The results for groups A-E were not statistically significant different, showing that despite the variations regarding background of the test users and the size of the groups the results were comparable. This proves both, the ease of use and the reproducibility of this test method with little intergroup variation. This test setup represents a new approach to standardize a subjective assessment such as the evaluation of handling properties of dental composites.
<table>
<thead>
<tr>
<th>Group</th>
<th>Background and affiliation</th>
<th>N=</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Dentists, Internal Clinic IvoclarVivadent, Schaan, Liechtenstein</td>
<td>5</td>
</tr>
<tr>
<td>B</td>
<td>General Practitioners, Germany</td>
<td>7</td>
</tr>
<tr>
<td>C</td>
<td>Dentists, University Heidelberg, Germany</td>
<td>8</td>
</tr>
<tr>
<td>D</td>
<td>General Practitioners, Eastern Europe</td>
<td>31</td>
</tr>
<tr>
<td>E</td>
<td>General Practitioners and KOL, USA</td>
<td>12</td>
</tr>
</tbody>
</table>

**Definition of overall handling according to the mean of the overall handling-score obtained in the test set-up**

<table>
<thead>
<tr>
<th>Classification</th>
<th>Mean for overall handling score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent/Very Good Handling</td>
<td>1.00 - 1.49</td>
</tr>
<tr>
<td>Good Handling</td>
<td>1.50 - 2.49</td>
</tr>
<tr>
<td>Satisfactory/Poor Handling</td>
<td>2.50 - 3.49</td>
</tr>
<tr>
<td>Unacceptable Handling</td>
<td>3.50 - 4</td>
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</tbody>
</table>
**The Color Stability of Composite Resins With and Without Periodic Brushing**

Berna Sadioglu¹, Murat Can Ersen², Irem Kaya¹, Nevin Cobanoglu¹

¹Restorative Dentistry, Selcuk University Faculty of Dentistry, Konya, Turkey, ²Restorative Dentistry, Selcuk University Faculty of Dentistry, Konya, Yeni İstanbul, Turkey

**Objectives**
The aim of this study is to compare the color stability of composite resins with and without periodic brushing during staining.

**Methods**
Disc-shaped samples were prepared from 4 different composite resins (Filtek Ultimate(FU), Omnichroma(OMC), Estelite Sigma Quick(EQ), Nova Compo(NC)). The samples were soaked in water for 24 hours, the first shade determinations were made (baseline), and each composite resin samples were divided into 3 groups (n=10): 1. Control; Soak in water for 5 days. 2. Periodic Brushing(PB); Brushing with toothpaste for 40 seconds at the end of every 24 hours and soaking in coffee for 5 days. 3. Non-Periodic Brushing(NPB); Brushing with toothpaste after soaking in coffee for 5 days. After 5 days, the colors measurements were repeated before brushing(staining) and after brushing(brushing). Color change values were calculated using the Baseline-Staining(ΔE1) and Baseline-Brushing(ΔE2) values. One-way ANOVA and Tukey's post-hoc test were used for statistical analysis. (α = 0.05).

**Results**
There was no statistically significant difference between the ΔE1 values of the PB and NPB groups for each composite resin. There was no statistically significant difference between the ΔE2 values of the composite resins except NC in both the PB and NPB groups. In both groups, the NC composite resin had a statistically significantly lower ΔE2 value. After brushing, the L value of the composite resins, except for NC, was found to be statistically significantly lower.

**Conclusions**
Periodic brushing during the staining period did not affect the color change value of any composite resin. After brushing, there was no difference in color stability of composite resins except NC in both PB and NPB groups. The best color stability was found for NC.

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**Spatial Polymerisation Characteristics of Bulk Fill Materials Cured With High Irradiance**

Mohammed Hadis, William Palin

School of Dentistry, University of Birmingham, Birmingham, United Kingdom

**Objectives**
To investigate the spatial and temporal polymerisation characteristics of “bulk fill” (BF) resin composites cured with high irradiance (3400 mW/cm²) and short exposure times (3 s).

**Methods**
“High energy” BFs (HBFs; Power Flow and Power Fill) and conventional resin composites (CRC; Tetric EvoFlow and Tetric EvoCeram) were cured (Bluephase Powercure; 3s turbo and 10s high mode (Ivoclar-Vivadent) in black nylon cylindrical-shaped moulds (12mm diameter; 2 or 4mm thickness). Spatial degree of conversion (DC) was mapped immediately after curing using a FTIR-microscope (Nicolet iN10 MX, Thermo-Scientific; ATR mode) in 250mm increments on the non-irradiated surface. Beam-profile and spectral irradiance of the curing light was measured using a CCD camera (SP620, Ophir Spricon) and mini UV-Vis spectrometer (STS, Ocean Optics).

**Results**
A significantly higher irradiance and lower radiant exposure was delivered using the turbo mode compared with the high mode (3137 ± 2mW/cm², 9.4 J/cm² and 1221 ± 2mW/cm², 12.2J/cm²). Both modes exhibited comparable beam profiles. DC was dependent on curing mode, material type and thickness (p<0.05). At 2mm thickness with high mode exposures, CRC materials exhibited significantly higher DC compared with HBF materials (CRC: 62.74%; HBF: 48.53% for PowerFill and 40.55% for PowerFlow; p<0.05). For HBF, comparable DC was measured at 4mm thickness using turbo mode compared with 2mm specimens using high mode. DC maps demonstrated relatively homogenous curing profiles for all high viscosity materials. Flowable materials exhibited lower DC around the periphery of the samples.

**Conclusions**
DC was affected by material thickness, material type and exposure mode. High energy photopolymerisation requires appropriate light optics and materials chemistry design to achieve homogenous spatial cure for thick light curable materials.
Temperature Increase During Polymerization in Short Fiber-Reinforced Composites.
Anna-Maria Le Bell-Rönnlöf1, Henrik Paatsamala1, Timo Närhi2, Pekka Vallittu1, Lippo Lassila2
1University of Turku, Turku, Finland, 2Turku Clinical Biomaterials Centre-TCBC, University of Turku, Turku, Finland

Objectives The aim of this in vitro study was to investigate the temperature increase during polymerization of composites measured at the bottom of a test cavity.

Methods Flowable short fiber-reinforced composite (SFRC) everXFlow (Bulk) and packable composite G-ænial Posterior (P-A3) (GC) were used in this study. Three groups with 5 samples were tested: everXFlow (Bulk) 4mm, everXFlow (Bulk) 2mm + G-ænial Posterior (P-A3) 2mm and G-ænial Posterior 2mm+2mm. Composites were applied in standardized cylindrical cavities (diameter=4mm, height=4mm) and each layer was light-polymerized 20s (3M ESPE Elipar DeepCure-L) in close contact with the cavity. Temperature change was measured with a heat sensor (PeakTech Type K Thermocouple) which was positioned at the bottom of the cavity. Empty cavity and a cavity filled with polymerized composite (everXFlow Bulk) served as positive and negative controls. In all groups temperature changes and heat peaks were recorded.

Results The results are presented in the Table.

The difference in measured mean temperatures at the first heat peak was statistically significant among all groups (p<0.05). At the second heat peak no significant differences were observed between the groups (p>0.05). The highest heat peak 53.7°C was observed in the empty cavity. In the group of 4mm flowable short fiber-reinforced composite everXFlow (Bulk) a mean temperature of 48.4°C at the first heat peak was measured. In the group of 2mm+2mm packable composite G-ænial Posterior (P-A3) the lowest mean temperature 36.8°C was observed at the first heat peak.

Conclusions The temperature rise from the light-curing device is considerably larger than that of the composites during polymerization. Composites in cavities act as an isolating layer that may protect the surrounding tooth/pulp structure.

The measured mean temperatures (°C) (±SD) at the first (T1) and the second (T2) heat peaks.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-ænial Posterior 2mm+2mm</td>
<td>36,8 (0.6)A</td>
<td>38,2 (0.8)A</td>
</tr>
<tr>
<td>Polymerized everXFlow (negative control)</td>
<td>41,1 (0.2)B</td>
<td></td>
</tr>
<tr>
<td>EverXFlow Bulk 2mm + G-ænial Posterior 2mm</td>
<td>44,7 (1.4)C</td>
<td>37,0 (1.1)A</td>
</tr>
<tr>
<td>EverXFlow Bulk 4mm</td>
<td>48,4 (1.8)D</td>
<td></td>
</tr>
<tr>
<td>Empty cavity (positive control)</td>
<td>53,7 (1.0)E</td>
<td></td>
</tr>
</tbody>
</table>

Different superscript letters indicate statistical difference (p<0.05, Tukey).
Physical Properties of a new one-Shade Flowable Bulk-Fill Composite
Janine Schweppe¹, Andreas Utterodt², Christoph Meier², Michael Eck², Kurt Reischl²
¹Global Scientific and Clinical Affairs, Kulzer GmbH, Hanau, Germany; ²R&D, Kulzer GmbH, Wehrheim, Germany

Objectives The purpose of this study was to compare flexural strength (FS), shrinkage stress (SS), depth of cure (DoC), radiopacity, two-body- and three-body-wear of a novel one-shade flowable bulk-fill composite to other bulk-fill composite materials.

Methods The tested composites were Surefil SDR flow+ (SDRF, Universal, Dentsply Sirona), Filtek ONE Bulk Fill (FOBF, A1, 3M), Tetric EvoCeram Bulk Fill (TECB, IVA, Ivoclar Vivadent), Tetric EvoFlow Bulk Fill (TEFB, IVA, Ivoclar Vivadent) and the new Venus Bulk Flow ONE (VBFO, ONE, Kulzer).
FS was measured according to ISO 4049 in a universal testing device after 24h water-storage. DoC was investigated following ISO 4049: All samples were light-cured from the top, the soft composite was scraped off and the height of the remaining composite cylinder was measured and divided by 2. Radiopacity was compared to an Aluminum step following ISO 4049. SS was tested using a photoelastic measurement after 24h water-storage. Wear was investigated by measuring wear depth after 200k cycles in a 2-body-wear chewing simulation and after 300k cycles in a 3-body-wear ACTA device (poppy seed).

All samples were light cured for 20s by a Translux Wave light curing unit.
Statistical analysis was performed by ANOVA, followed by LSD test (p=0.05).

Results The results are depicted in the table.

Conclusions Within the limitations of this study, it can be summarised that the new one-shade flowable bulk-fill VBFO shows good physical properties which enable bulk filling up to 4mm in posterior teeth without the need of an additional occlusal capping layer.

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>SDRF</td>
<td>116.2±16.3B</td>
<td>6.65±0.17A</td>
<td>202.8±12.5D</td>
<td>32.3±2.1B</td>
<td>4.9±0</td>
<td>312</td>
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<tr>
<td>FOBF</td>
<td>159.3±18A</td>
<td>7.74±0.86B</td>
<td>184.5±10.4C</td>
<td>31.7±1.2B</td>
<td>3.75±0</td>
<td>281</td>
</tr>
<tr>
<td>TECB</td>
<td>115.4±14.4B</td>
<td>6.96±0.05A</td>
<td>188.2±10.4C</td>
<td>43.3±1.2C</td>
<td>4.1±0.1</td>
<td>294</td>
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<tr>
<td>TEFB</td>
<td>113.8±10.8B</td>
<td>7.27±0.1AB</td>
<td>165.1±16.2B</td>
<td>29.9±0.7A</td>
<td>4.6±0</td>
<td>259</td>
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<tr>
<td>VBFO</td>
<td>120±8B</td>
<td>6.76±0.13A</td>
<td>140.3±15A</td>
<td>46.7±2.1D</td>
<td>4.2±0.1</td>
<td>299</td>
</tr>
</tbody>
</table>

No significant differences between materials with same letters in a column were found.
Light Attenuation Effect: Depending on Different Thicknesses of Resin-Matrix Ceramic Material

Irem Okten¹, Merve Uctasli²

¹Department of Restorative Dentistry, Faculty of Dentistry, Ege University, Izmir, Turkey, ²Department of Restorative Dentistry, Faculty of Dentistry, University of Gazi, Ankara, Turkey

Objectives To evaluate light penetration through different thicknesses of resin-matrix ceramic material by means of mean irradiance and time required to reach 16 J/cm².

Methods Ten test specimens (n=10) with different thicknesses as 2.00mm, 1.5mm, 1.00mm and 0.5mm were prepared using resin-matrix ceramic material (CERASMART270 A1HT, GC). Lower surfaces of test specimens sandblasted by means of 50µm aluminum-oxide particles applied for 10s at a working distance of 10mm and a pressure of 0.28MPa. Upper surfaces of test specimens were polished (DiacompPlusTwist and DiaPolisherPaste, GC). Light penetration through different thicknesses of test specimens were measured with MARC Resin Calibrator (BlueLight Analytics Inc.) applying 10s light irradiation (Elipar DeepCure-S LED Curing Light, 3M-ESPE) and measured by means of mean irradiance and time required to reach 16 J/cm². Statistical analysis was performed using one way ANOVA at 95% confidence level(α=0.05).

Results The mean and the standard deviation of test groups of mean irradiance (mW/cm²): only 10s light irradiation without restorative materials (control group), light irradiation through 2.00mm, 1.5mm, 1.00mm and 0.5mm thick resin-matrix ceramic materials were 1976.6 ± 52.7 (control group), 329.8 ± 9.80, 462.7 ± 33.56, 601.2 ± 16.47 and 989.4 ± 25.45, respectively. The mean irradiance measurements showed statistically significant difference between the control group and different thicknesses of the test groups(p<0.05).

The highest and the lowest measurements of test groups for time required to reach 16 J/cm²(s): only 10s light irradiation without restorative materials (control group), light irradiation through 2.00mm, 1.5mm, 1.00mm and 0.5mm thick resin-matrix ceramic materials were 8.62-7.02; 53.04-45.46; 40.26-32.22; 27.6-24.38; 16.31-15.4 seconds, respectively. The highest and the lowest measurements of test groups showed statistically significant difference between the control group and different thicknesses of the test groups(p<0.05).

Conclusions When the thickness of resin-matrix ceramic material was increased light penetration was decreased. Other means, the mean irradiance was increased, time required to reach 16 J/cm² was decreased.

Right Choice of Finishing for the Smoothest Surface of Amalgam-Replacement-Materials

Alexander Behlau¹, Isabelle Dinslaken², Thomas Schmid¹, Michael Payer¹, Gerd Leitinger¹, Lumnije Kquiku-Biblekay¹, Katharina Hanscho¹, Karl Glockner¹

¹Medical University of Graz, Graz, Austria, ²Bundeswehr University Munich, Munich, Germany, ³Technical University of Graz, Graz, Austria

Objectives There is a growing endeavour to improve conservative dentistry. At least since the amalgam ban of the European Union in 2017 for children under fifteen, as well as for pregnant and breast-feeding women, there is a growing need to investigate the longevity of potential amalgam-replacement materials. A low surface roughness is a key quality for the longevity of dental fillings. The aim of the present study was to investigate the influence of different finishing methods for varying amalgam-replacement materials on surface roughness over time.

Methods In the present in-vitro study Cention Forte, DeltaFil, Equia Forte HT, IonoStar Molar and Ketac Universal were examined with regard to their surface roughness (Sa). For this purpose, standardized metal printed molds were filled with the respective filling materials. After extrusion the samples (n=300) were analysed using a noncontact profilometer (InfiniteFocus, Bruker Alicona). Different finishing methods (n=10 per group) were compared: (1) without finishing (control group), (2) arkansas bur, (3) diamond bur, (4) tungsten carbide bur, (5) Soflex discs in descending grit size and (6) coarse Soflex discs in combination with a silicone polisher. Samples were analysed at three time points: (A) after finishing, (B) after 30 days and (C) after an aging simulation using thermocycling.

Results It could be shown that surface roughness varies between different filling materials and due to varying finishing methods. For example, the surface roughness of a glass-ionomer cement was lowest using soflex discs, while arkansas bur revealed the smoothest surface of an alkasite. Except for DeltaFil, surface roughness increased over time.

Conclusions The present study extend our knowledge for the choice of the optimal finishing method for different amalgam-replacement materials to produce the smoothest surface. The study gives hints for practitioners who want to use amalgam-replacement materials.
Development and Characterization of Bioactive Fiber-Reinforced Composite

Sufyan Garoushi, Pekka Vallittu, Lippo Lassila

Biomaterials Science, University of Turku, Turku, Finland

Objectives This study aimed to develop and characterize a restorative system combining bioactive fiber-reinforced flowable resin composite (Bio-SFRC) and dentin activator solution made of poly(acrylic acid) (PAA) with high molecule weight. In addition to evaluate the interface and remineralization potential between the Bio-SFRC and dentin.

Methods Certain mechanical properties (flexural strength and fracture toughness) of experimental Bio-SFRC in comparison with commercial biostable (G-aenial Flo X) and ion-releasing materials (ACTIVA-BioActive Base/Liner and Fuji II LC) were assessed (n=8/group). Calcium release at different time-point was measured during the first two weeks by using a calcium ion selective electrode. Surface analysis of composites after being stored in simulated body fluid (SBF) was investigated by using SEM/EDS. Dentin discs (n=50) were prepared from extracted sound teeth and demineralization was simulated by acid etching. For testing the remineralization on the top surface of demineralized dentin, discs (n=30) were either surface treated with PAA activator or without (control) before immersion in SBF for one week. For testing the interface between the investigated materials and demineralized dentin, materials were applied on discs (n=5/per material) and light-cured before immersion in SBF for two weeks. SEM/EDS was used to evaluate the microstructure of dentin on the top surface and at interface with composites after being stored in SBF.

Results Bio-SFRC showed higher fracture toughness (1.6 MPa m^{1/2}) compare to commercial tested materials (p<0.05). Accumulative calcium release after two weeks from Bio-SFRC (12 mg/l) was much higher than other tested ion-releasing materials (= 4.3 mg/l). Mineralization formation was clearly seen at the interface between activated dentin and Bio-SFRC. None of the commercial tested materials showed signs of mineralization at the interface and dentinal tubules remained open.

Conclusions Developing such reinforced flowable resin composite and PAA might offer the potential for remineralization at the interface and inside the organic matrix of demineralized dentin.
Viscoelastic Behavior of Universal Chromatic Micro-Hybrid Resin-Based Composites After Accelerated Aging.
Andrei C. Ionescu¹, Nicoleta Ilie²
¹Biomedical, Surgical, and Dental Sciences, University of Milan, Milan, MB, Italy, ²Restorativ, LMU, Munich, Germany

Objectives This study characterized the mechanical behavior of 6 conventional light-cured resin-based composites (RBCs) and 3 universal chromatic ones designed to adapt to sundry clinical situations esthetically.

Methods Quasi-static and viscoelastic parameters were evaluated at a clinically relevant frequency (1 Hz) using an instrumented indentation test with a Dynamic Mechanical Analysis (DMA) module. The latter investigated both elastic and viscous material responses simultaneously by superimposing a small oscillating force (1 Hz) on a quasi-static force of 1 N. The amplitude was adjusted to 5 nm to keep the deformation in the linear viscoelastic regime. Ten measurements were performed on each of 5 specimens per RBC. Specimens were aged 24h, 3M, 6M, 6M + 72h in 75% EtOH. Multifactor analysis of variance was applied after verifying the normality of distribution to compare the parameters of interest (Martens, Vickers, and indentation hardness; elastic and total indentation work; creep, indentation depth; storage, loss, and indentation moduli; loss factor).

Results A multivariate ANOVA showed a significant effect (p < 0.001) of RBC and aging factors and a significant interaction between factors on all measured parameters. Structural particularities of the filler systems are directly reflected in the mechanical behavior of the tested RBCs. BFP (Table 1) showed the lowest overall mechanical parameters, while VD showed the highest ones. Interestingly, aging procedures did not deteriorate the mechanical characteristics of BFP and VD.

Conclusions Based on the performed characterization, universal chromatic RBCs exhibited comparable behavior to conventional micro-hybrid RBCs. BFP and VD outstandingly withstood aging procedures.

RBCs tested in the present study. PPF, pre-polymerized filler.

<table>
<thead>
<tr>
<th>Code</th>
<th>RBC</th>
<th>Manufacturer</th>
<th>Shade</th>
<th>Monomer</th>
<th>Filler</th>
<th>Filler wt/vol</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF</td>
<td>Admira Fusion</td>
<td>VOCO</td>
<td>A3.5</td>
<td>Ormocer</td>
<td>SiO2</td>
<td>84/n.s.</td>
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<tr>
<td>AFX</td>
<td>Admira Fusion x-tra</td>
<td>VOCO</td>
<td>U</td>
<td>Ormocer</td>
<td>SiO2</td>
<td>84/n.s.</td>
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<td>BEG</td>
<td>Brilliant EverGlow</td>
<td>Coltene</td>
<td>A3/D3</td>
<td>Bis-GMA TEGDMA</td>
<td>PPF, SiO2, Ba-Al2O3-SiO2</td>
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<td>BFP</td>
<td>Beautifil Flow Plus F03</td>
<td>Shofu</td>
<td>A3</td>
<td>Bis-GMA TEGMA</td>
<td>S-PRG, B2O3-F-Al2O3-SiO2</td>
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<tr>
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<td>Beautifil II LS</td>
<td>Shofu</td>
<td>A3</td>
<td>UDMA Bis-MPEPP Bis-GMA TEGDMA</td>
<td>PPF, B2O3-F-Al2O3-SiO2</td>
<td>83/69</td>
</tr>
<tr>
<td>EBF</td>
<td>Ecosite Bulk Fill</td>
<td>DMG</td>
<td>U</td>
<td>Bis-GMA DMA</td>
<td>Ba-Glass</td>
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<td>OC</td>
<td>Omnichromma</td>
<td>Tokuyama</td>
<td>one</td>
<td>UDMA TEGDMA</td>
<td>SiO2, ZrO2 &amp; CF</td>
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<td>Kulzer</td>
<td>one</td>
<td>UDMA TCD-DI-HEA TEGDMA</td>
<td>BaAlF, SiO2</td>
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<td>Kulzer</td>
<td>one</td>
<td>UDMA TCD-DI-HEA TEGDMA</td>
<td>BaAlF, SiO2 &amp; PPF</td>
<td>75/59</td>
</tr>
</tbody>
</table>
Development of a Novel Resin-Modified Glass-Ionomer Cement With Improved Mechanical Properties

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Objectives Development of a novel RMGICs containing a ceramic (zirconia) reinforced glass ionomer cement powder and a liquid containing alternative monomers to hydroxyethyl methacrylate (HEMA), with improved mechanical properties and reduced water uptake compared to commercial materials.

Methods Powder of two commercial materials were used in this study. Powder 1: Amalgomer CR (AHL, UK) which contains a ceramic filler (zirconia) and powder 2: Fuji II LC (GC, Japan). These powders were mixed with two liquids, one containing HEMA and the other containing 50%/50% tetrahydrofurfuryl methacrylate (THFM) and HEMA, respectively. The powder and liquid were mixed and poured into PTFE moulds and light cured for 20 sec to produce samples. Fuji II LC (GC, Japan) was used as a control. Samples (n=6) were tested with respect to flexural strength (25mm, 2mm, 2mm), water uptake and desorption (15mm diameter, 1mm thickness). All materials were characterised using Fourier Transform Infrared Spectroscopy (FT-IR).

Results As confirmed by FT-IR, all materials showed evidence of polymerisation following irradiation for 20 seconds. All novel RMGICs showed improved flexural strength values compared to commercial Fuji II LC. Moreover, novel RMGICs containing ceramic reinforced powder (Amalgomer CR) showed significantly higher flexural strength compared to all other materials tested. Materials incorporating 50%THFM showed significantly lower water uptake compared to their counterparts incorporating 100% HEMA.

Conclusions Partially replacing HEMA with THFM reduced the water uptake of light-cured RMGICs. Furthermore, groups containing Amalgomer CR ceramic reinforced glass powder showed promising results with improved mechanical properties, which warrant further investigations.

In-Vitro Evaluation of Mouthwashes on Virus Infectivity and RNA Stability

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Objectives Reverse-transcription polymerase chain reaction (RT-PCR) is a commonly employed method for quantitative detection of SARs-CoV-2 and has been applied to clinical studies evaluating the activity of oral care products to reduce SARs-CoV-2 salivary load. The objective of this in-vitro study was to compare the viral-stability of a related coronavirus, Feline coronavirus (FCoV), measured by qRT-PCR with the viral-activity of FCoV, following treatment with two commercial mouthwashes in simulated oral plaque.

Methods Two mouthwash formulations (A -0.2% chlorhexidine/non-alcohol and B -IPMP/Zn/SLS) were tested for reducing viral-infectivity and viral-stability using a modified BS EN 16777:2018 and qRT-PCR respectively, compared to a water control. FCoV ATCC VR-989 was chosen as a surrogate for SARs-CoV-2. A virus/plaque mixture was applied on to a hydroxyapatite surface and allowed to dry. Test products were applied neat for contact time of 30-seconds and two-minutes. Products were tested in triplicate. QIAamp Viral RNA extraction and VetMax FIP dual IPC kit were used for qRT-PCR analysis.

Results For infectivity, mouthwash-A did not produce a significant reduction in the titre of FCoV when compared to water control (mean reduction at 30 sec: 0.39 log; at 2 min: 0.18 log). In contrast, Treatment-B produced a significant reduction in viral titre when compared to the water control (mean reduction at 30 sec: 2.55 log; at 2 min: 1.84 log). For viral-stability, no significant difference in the viral RNA levels were detected for either groups compared to control (mouthwash-A Ct=15.4; mouthwash-B Ct=15.0; water-control Ct=15.7).

Conclusions This in-vitro study demonstrates that oral mouthwashers can show different virucidal effects. However, qRT-PCR may not reveal differences in viral inactivation between treatments or water control, at least at relatively high viral loads. Viral RNA from inactivated viral particles may be confounding qRT-PCR results.
Analysis of Oral Microbiome in Bariatric Surgery Patients and in Mice

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Objectives Obesity and bariatric surgery induce systemic alterations that can be reflected at the oral cavity. The nature of these alterations is still not conclusive. This research aims to investigate the effect of obesity and bariatric surgery on the oral health status and oral microbiome in humans and in mice.

Methods The oral status of obese patients (pre-surgery, 1/2Y post-surgery) and lean volunteers was examined, and categorized accordingly to gingivitis, caries, and periodontitis. Patients’ oral plaque microbiome was explored using next-generation sequencing of 16S rDNA and assessed by analysis and statistical tests. Furthermore, 40 C57BL/6 mice were fed high-fat diet, and go through: experimental periodontitis (PERIO±), sleeve gastrectomy (SG±). Wight and glucose levels were monitored and oral and gut microbiome were collected. Maxillary jaws were harvested for alveolar bone volume estimation using μCT and for H&E and TRAP staining.

Results Microbial diversity was significantly higher in bariatric patients, and was increased post-surgery. Microbiome composition showed distinguished clustering trend between the three groups. Higher prevalence of Streptococcus and pseudomonas in control group, while Neisseria, Veillonella, Aggregatibacter-segnis and copnocytophaga were higher in obese patients. Interestingly, oral Bacteroidota/Proteobacteria ratio was the highest in the bariatric group indicating higher dysbiosis. In mice, systemic indicators and maxillary volume were significantly different between SG+/ PERIO+ to SG- /PERIO- groups. Oral microbiome diversity increased in SG+/PERIO+ group post-surgery compared to baseline and pre-surgery. The oral microbiome composition was significantly different between SG-/PERIO+, SG+/PERIO+. Sanguibacter oral abundance increased with obesity, while Alkanindiges-illinoisensis decreased. Streptococcus-equii and Paraburkholderia-bryophila decreased post-surgery. Higher Lachnospiraceae abundance in SG-/PERIO+ compared to SG-/PERIO-. Interestingly, oral gavage of P.g induced gut microbiome changes.

Conclusions All together, these results suggest that there is an association between oral health and obesity and that oral microbiome is affected by obesity, and weight loss after bariatric surgery.
Effects of Daily Antiseptic Treatment on Saliva-Grown Microcosm Biofilms
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1University Hospital Regensburg, Department of Conservative Dentistry and Periodontology, Regensburg, Bavaria, Germany, 2Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Regensburg, Bavaria, Germany, 3Department of Operative Dentistry and Periodontology, Center for Dental Medicine, University of Freiburg, Freiburg, Baden-Württemberg, Germany, 4Department of Conservative Dentistry and Periodontology, University Hospital Regensburg, Regensburg, Bavaria, Germany

Objectives Antiseptics are in widespread use in dental practice and included in lots of over-the-counter oral care products. However, the effects of routine antiseptic use on microbial composition of oral biofilms and on emergence of resistant phenotypes remain unclear. The aim of this study was to investigate ecological changes in mature saliva-grown microcosm biofilms upon twice daily application of CHX and CPC for a period of 7 days, and to evaluate whether suchlike multiple application of CHX or CPC selects for resistant phenotypes.

Methods In this study, saliva-grown microcosm biofilms of 4 healthy donors were cultured in the Amsterdam Active Attachment biofilm model for 3 days and treated twice daily with CHX or CPC for a period of 7 days. Ecological changes upon these multiple antiseptic treatments were evaluated by semiconductor-based sequencing of bacterial 16S rRNA genes and identification of amplicon sequence variants (ASVs). Culture-based approaches were used for colony forming units (CFU) assay, identification of antiseptic-resistant phenotypes using an agar dilution method and evaluation of their antibiotic susceptibilities.

Results CHX and CPC showed only slight effects on CFU and could not inhibit biofilm growth despite the twice daily treatment for seven days. Both antiseptics showed significant ecological effects on the microbial compositions of the surviving microbiota, whereby CHX led to enrichment of rather caries-associated saccharolytic taxa e.g., Lactobacillales or Neisseriales, and CPC led to enrichment of rather gingivitis-associated proteolytic taxa e.g., Fusobacteriales or Selenomonadales. At least one antiseptic-resistant taxon per donor was isolated on antiseptic-containing agar plates, which also exhibited phenotypic resistance to various antibiotics.

Conclusions These results highlight the need for further research into potential detrimental effects of antiseptics on the microbial composition of oral biofilms and on spread of antimicrobial resistance in the context of their widespread use in oral health care.
P299

Effects of CPC- and CHX-Containing Mouthwash in SARS-CoV-2 Positive Patients

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Objectives Since the beginning of the COVID-19 pandemic, preprocedural mouthwashes have been discussed as a potential measure to temporarily reduce intraoral viral load and infectivity in potentially SARS-CoV-2 positive individuals prior to aerosol-generating procedures. Particularly, the antiseptic cetylpyridinium chloride (CPC) has shown virucidal effects toward SARS-CoV-2 in vitro. Therefore, the aim of this randomized controlled clinical trial was to investigate the efficacy of a commercially available mouthwash containing CPC and chlorhexidine digluconate (CHX) at 0.05% each (PerioAid Active Control, Dentaid SL) in SARS-CoV-2 positive patients as compared to a placebo mouthwash.

Methods 61 Patients tested positive for SARS-CoV-2 with onset of symptoms within the last 72 hours were included in this study. Oropharyngeal specimens were taken at baseline, whereupon patients had to gargle mouth and throat with 20 mL PerioAid Active Control (test) or 0.9% NaCl (placebo) for 60 seconds. After 30 minutes, further oropharyngeal specimens were collected. Viral load was analyzed by RT-qPCR. Furthermore, infectivity of oropharyngeal specimens was analyzed via virus rescue in cell culture and quantified via determination of TCID50. Data were analyzed non-parametrically (Mann-Whitney tests; alpha=0.05).

Results Viral load slightly, but significantly decreased upon gargling in the test group, but not in the placebo group. Viral infectivity as measured by TCID50 also significantly decreased in the test group, whereas the effect in the placebo group was minor. Furthermore, patients with a vaccine booster exhibited significantly decreased infectivity at baseline as compared to those without vaccine booster.

Conclusions This clinical trial shows that a preprocedural mouthrinse with a commercially available mouthwash containing CPC and CHX could significantly reduce the viral load and infectivity in SARS-CoV-2 positive patients.

Further studies are needed to establish whether the observed reductions in viral load and infectivity could translate into a clinically useful reduction in the risk of transmission of SARS-CoV-2.

P400

In Vitro Evaluation of Antiviral Effects of Oral Care Products

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Objectives to compare the efficacy of oral care products in inactivating a surrogate of Covid-19 virus in vitro.

Methods Five toothpastes and six mouthwashes were evaluated using ASTM E1052–20 versus Vaccinia virus VRV–1549 Elstree strain in Vero cells (as a standard surrogate for Beta Coronavirus, Siddharta et al (2017) J Infect Dis 215: 902–6). Toothpastes were tested as 25% w/w slurries in synthetic saliva, and mouthwashes tested at 80% v/v in synthetic saliva to represent in use concentrations. Treatments were carried out for 30s, 1min or 2min. The Tissue culture infectious dose/50 (TCID50) of surviving virus was determined, and compared with untreated control and formaldehyde as negative/positive controls respectively. Toothpastes evaluated were all sodium dodecylsulfate (SDS)/fluoride-based toothpaste formulations, with active components as follows: stannous fluoride; stannous fluoride/zinc chloride; IPMP/zinc chloride; zinc chloride; no additional active. Six mouthwashes evaluated included the following actives: Chlorhexidine (at 0.06 or 0.20% w/w); Cetylpyridinium chloride (CPC); Ethanol/Essential Oils; IPMP/ zinc chloride /SDS; hydrogen peroxide; CPC/zinc chloride; povidone/iodine.

Results Treatment with all 5 toothpastes resulted in complete inactivation of detectable virus (>4 log10 or >99.99% inactivation), at all time points tested. For mouthwashes, the IPMP/Zn/SLS mouthwash produced complete inactivation, as did the CPC-only mouthwash and the Povidone-Iodine formulation; peroxide and ethanol/EO mouthwashes showed >2log10 / >99% inactivation at 2min; CPC/Zn mouthwash showed 1.95log10/98% inactivation. Chlorhexidine mouthwashes showed poor (<1log10/<90%) inactivation. Inactivation was superior for SLS containing products (either toothpastes or mouthwashes) and for CPC only mouthwash and iodine products.

Conclusions Oral care product efficacy against surrogate Covid viruses varied sharply, dependent on compositional differences.
Compared. Digital evaluation was provided via the CANVAS online learning platform. This 5 stage linear examination had little effect on student performance. The mean score for the electronic assessment was completely standardised with no intra

Conclusions

undi

understanding tasks in the face

remotely and securely. When compared, both the electronic and traditional assessments had many similarities including complexity, standard-setting and blue-printing. Both OSCEs assessed students’ history-taking, diagnostic and treatment planning skills. Differences included the need for assessment orientation training with CANVAS prior to the examination. Due to the international cohort of students undertaking the course, the online examination was time-limited over a 24 hr period to accommodate the wide dispersion of candidates.

Results

format changes to the examination had little effect on student performance. The mean score for the remote assessment was 67.4% (SD=7.17) compared to 66.8% (SD=5.84) for the face-to-face assessment undertaken 1 year previously. Although students performed to a similar standard across the examinations, intra-station variations were observed between the 2 assessments. Students performed more strongly in history-taking tasks in the face-to-face examination. In the electronic examination students performed better at special investigation interpretation tasks.

Conclusions

The Canvas platform provided suitable means to assess students during the COVID restrictions. The electronic assessment was completely standardised with no intra-station variation. As with all assessments, the importance of robust design and standard setting is crucial.
Dentinal Tubules Penetration of Bioceramic and Conventional Sealers: SEM Analysis

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Objectives
The obturation of the endodontic space represents the final phase of a root canal therapy and, like the previous phases such as instrumentation and cleansing, helps to determine its success. The aim of this work is to evaluate, by scanning electron microscope analysis, the influence of bioceramic and conventional sealers on penetration depth and adaptation of dentinal tubules, both used in association with a "warm" obturation technique (continuous wave of condensation) and a "cold" obturation technique (single cone).

Methods
Forty canines extracted for periodontal reasons were selected for the study. According to the type of sealer and to the root canal filling technique used, the specimens were divided into 4 groups: single cone technique with conventional zinc oxide-eugenol-based sealer (Pulp Canal Sealer); continuous wave of condensation technique with zinc oxide-eugenol-based sealer; single cone technique with tricalcium silicate sealer (BioRoot RCS); continuous wave of condensation technique with tricalcium silicate-based sealer.

Results
Continuous wave of condensation in association with bioceramic sealers showed deeper root dentin penetration. Better adaptation of the sealer to apical root dentin was achieved with continuous wave of condensation in comparison to the other three experimental groups.

Conclusions
From the analysis of the results it can be concluded that with the same filling technique, bioceramic sealers seems to be more performing than the conventional one: it exhibited a greater degree of penetration and a higher quality of adaptation. In addition, the association of bioceramic sealers with the continuous wave of condensation seems to be particularly promising in improving the quality of the seal, in terms of depth of penetration and quality of adaptation.

Numerical Simulation of Debonding During Composite Polymerization

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Objectives
The study aims to apply fracture mechanics principles in finite element analysis to simulate the bond deterioration in class I cavities.

Methods
The entire modeling and simulation were conducted using the open-source software Salome-Meca. Cavities of different dimensions, with diameters ranging from 2 to 6 mm and heights of either 1 or 2 mm, were created in axisymmetric tooth models. Polymerization of the composite was simulated by thermal analogy and temporal development of the elastic modulus. The damage at the adhesive interface was determined based on the interfacial fracture toughness and tensile bond strength reported in the literature. Maximum principal stress and displacement field were visualized in each model. For comparison, one additional model, with perfect bonding and a linear elastic adhesive layer, was created as the conventional approach.

Results
The results indicated the initiation of debonding from occlusal margin. Debonding altered both magnitude and direction of the post-polymerization displacement field, and the stress release resulting from debonding can be observed. The highest value of maximum principal stress reported was lower in our fracture-mechanics based model (104 MPa in the cavity of 6 mm diameter and 2 mm height, versus 425 MPa reported in the conventional model). The depth of debonding increased with cavity diameters and volumes, while there was an inverse relationship between C-Factors and the extent of debonding.

Conclusions
The study demonstrates the adhesion failure during simulated composite polymerization. Manifestations of debonding, including changes in displacement and internal stress, can be revealed with the novel approach. Bond deterioration can be predicted by cavity volumes and diameters. To reduce debonding, it is recommended not to bulk-cure composites in cavities of large diameters, even if their thickness is between 1 mm and 2 mm.
Immediate Bonding Property of Dual-Cure Resin Cement in Light-Cure Mode

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Objectives The dual-cure resin cement is often cured by light-curing mode since it can shorten a clinical time. However, there are clinical cases which are difficult to sufficiently light-cure such as at approximal area or under thick restoration, and its initial bonding property might be adversely affected. As a result, the longevity of the restorations may become short. To investigate this issue, we evaluated immediate bonding property of resin cements under thick restoration to simulate insufficient light curing.

Methods Three resin cement systems (G-CEM ONE (GCO, GC), RelyX Universal (RXU, 3M) and PANAVIA SA Cement Universal (PSU, Kuraray)) were used in this study. Specimens of bovine anterior tooth dentin were embedded with acrylic resin. Exposed flat dentin surface was finished with 600-grit SiC paper. Bonding area (3.0mm diameter) and cement thickness (0.1mm thickness) were defined by plastic-tape. Sand-blasted CERASMART270 plate (1.0, 2.0 and 3.0mm thickness) was adhered to dentin by each resin cement system and light irradiated 10mm away from specimen according to the manufacturer’s instructions. Specimens were stored in 37°C 100%R.H. for 5 minutes. Tensile bond strength (TBS) was measured by SHIMADZU AG-1. [Crosshead-speed 1mm/min, n=8, statistically analyzed (2-way ANOVA followed by Tukey post-hoc test, α=0.05)]

Results TBS of RXU and PSU were decreased with increase of CERASMART270 plate thickness. TBS of GCO was higher than the other products and showed no significant difference among tested thickness of CERASMART270.

Conclusions TBS of RXU and PSU was affected by insufficient light curing. On the other hand, GCO showed high immediate bonding property even insufficient light curing. This may suggest that GCO provide stable performance on clinical situation. This result is brought by the chemical initiator in G-CEM ONE Adhesive Enhancing Primer which accelerates the chemical cure of G-CEM ONE even without light-curing.

Result of tensile bond strength test

<table>
<thead>
<tr>
<th>TBS (S.D.) MPa</th>
<th>CERASMART270 1mm</th>
<th>CERASMART270 2mm</th>
<th>CERASMART270 3mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCO</td>
<td>12.8 (2.5) a</td>
<td>13.8 (3.4) a</td>
<td>12.5 (2.0) a</td>
</tr>
<tr>
<td>RXU</td>
<td>11.9 (2.9) ab</td>
<td>8.8 (1.7) bc</td>
<td>5.8 (1.3) cd</td>
</tr>
<tr>
<td>PSU</td>
<td>5.8 (1.6) cd</td>
<td>3.3 (0.9) de</td>
<td>2.2 (0.5) e</td>
</tr>
</tbody>
</table>

The same letters indicate non-significant differences.

Inlay Cavity Design and Bonding Effect on Tooth Fracture Load

Topias Yli-Urpo, Lippo Lasilla, Pekka Vallittu, Timo Närhi

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Objectives The aim of this study was to investigate the effect of indirect MOD inlay’s cavity bottom curvature geometry on the fracture load of the restored tooth. Test was performed with the state-of-the-art inlay bonding protocol and with intentionally deteriorated bonding to demonstrate the effect of inlay’s bonding on the fracture load.

Methods Forty-eight extracted intact molar teeth were selected and randomly divided into 4 groups (n=12/group): G1: Bonded inlay with square-shaped cavity design; G2: Non-bonded inlay with square-shaped cavity design; G3: Bonded inlay with curved cavity design; G4: Non-bonded inlay with curved cavity design. Cavities were prepared, scanned, and inlays milled from Cerasmart (GC) blocks using CAD/CAM technology. In bonded teeth, restorations were cemented adhesively while in non-bonded teeth, bonding was intentionally deteriorated with n-hexane wax. Restored teeth were attached to PMMA blocks and loaded occlusally using steel ball (diameter 5.5mm) in air from the occlusal surface along the long axis of the tooth. Ultimate fracture load (N) was recorded and type of the fracture was visually determined. Statistical analysis was performed by the means of ANOVA.

Results Mean ultimate fracture load values within groups: G1: 1283N, G2: 778N, G3: 1658N and G4: 675N. ANOVA revealed that bonding had a significant influence on tooth strength (G1 compared to G2: p=0.0022; G3 compared to G4: p=0.0003), but cavity design didn’t have significant influence (G1 compared to G3: p=0.1910; G2 compared to G4 p=0.8075). Fracture type analysis showed that in the groups of deteriorated bonding inlay and tooth fractured separately whereas with well bonded inlays fractures were commonly found both in the tooth substance and restoration.
**Conclusions** According to this study, curved cavity geometry reduced destructive fractures compared to the teeth with square-shaped cavity geometry. Properly bonded inlays showed higher load values than those where the bonding was deteriorated.

**Fig. 1** Schematic representation of square-shaped and curved tooth cavity designs, restoration, and metal ball contact with the restoration in loading test.

**Fig. 2** Mean ultimate fracture load and standard deviation (N) for each group. Group 1 (bonded inlay with right angle cavity design), Group 2 (non-bonded inlay with right angle cavity design), Group 3 (bonded inlay with curved cavity design), Group 4 (non-bonded inlay with curved cavity design).
Aesthetic Properties of Newly Developed 2-Step Self-Etch Adhesive “G2-BOND Universal”

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Objectives Generally, 2-step self-etch adhesives have high bond strength because of its thick bonding layer. They comprise PRIMER and BOND. BOND which has hydrophobic composition plays a role in forming thick and rigid bonding layer. However, adhesives have been formulated with HEMA to obtain compatibility with tooth substance, this may tend to have negative impact on durability and aesthetic property due to its hydrophilicity. Our new material “G2-BOND Universal” comprises “1-PRIMER” and “2-BOND”, both of which are HEMA-free composition. The purpose of this study was to find impact of HEMA-free composition on long-term durability and aesthetic property by evaluating physical properties of bonding layer after 6 months soaking in water.

Methods Two adhesives were selected, 2-BOND from G2-BOND Universal (GC, G2B), and BOND from Clearfil SE BOND 2 (Kuraray, SE2) which contains HEMA. These were evaluated by water absorption test and discoloration test. The specimens for both tests were prepared with reference to ISO 4049:2019 water sorption test and soaked in water at 37 degreeC for 6 months (n=5). Water absorption test was conducted by measuring the weight of specimens before and after soaking and calculated water absorption from weight difference. Regarding discoloration test, color value of specimens before and after soaking were measured with spectrometer (CM-3610d, MINOLTA) and calculated color change as ΔE*ab. All results were analyzed by one-way ANOVA (p<0.05).

Results G2B showed significantly lower water absorption and color change than SE2 after 6 months soaking. These results indicated G2B has possibility to be durable bonding layer which can contribute to aesthetic treatment because of its HEMA-free composition.

Conclusions In this study, G2B exhibited low water absorption and color change after long-term water soaking. Therefore, it’s expected that G2B, which has HEMA-free composition, has long-term durability and aesthetic property of bonding layer, which may have clinical advantages.

<table>
<thead>
<tr>
<th>Material</th>
<th>Water absorption [μg/mm3]/n=5</th>
<th>Discoloration [ΔE*ab value]/n=5</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2-BOND Universal 2-BOND (GC)</td>
<td>54.7 (1.2) a</td>
<td>2.2 (0.8) A</td>
</tr>
<tr>
<td>Clearfil SE BOND2 (Kuraray)</td>
<td>85.4 (0.8) b</td>
<td>16.2 (0.3) B</td>
</tr>
</tbody>
</table>

Same alphabet means no significant difference (p<0.05).
Evaluation of Bonding Performance of Self-Adhesive Composite Resin
Yuki Kasai, Kyosuke Hirano, Futoshi FUsejima
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Objectives The flowable composite resin products with self-adhesive function to tooth structure has been launched in to simplify the bonding procedure. There have been several reports about its bonding performance. In this study, we evaluated initial shear bond strength (SBS) and bond durability of self-adhesive composite resin products including our trial material, SACR-01.

Methods SACR-01(GC), FIT SA (SHOFU), Fusio (Pentron), Vertise Flow (Kerr) and Constic (DMG) were evaluated. Bovine enamel and dentin surface were polished with 400-grit SiC paper. SACR-01 was applied to prepared tooth surface, agitated for 10 seconds, and then irradiated with light for 10 seconds. Other composite resin was applied according to the manufacture’s instruction. Specimens were stored in water at 37 deg C 24 hrs. (24h) or subject to thermal cycle (TC10000; 5-55 deg C, 10000 times, afterTC), and then specimens were subjected to shear bond strength (SBS) test [n=5, statistically analyzed (two-way ANOVA followed by Tukey post-hoc test, p<0.05)].

Results SACR-01 showed significantly higher SBS to both enamel and dentin than other products in afterTC (Table 1). There was no significant difference of SBS for SACR-01 between 24h and afterTC. On the other hand, SBS on dentin of FIT SA in afterTC significantly decreased that it in 24h, and SBS on dentin of Fusio, Vertise Flow, and Constic in afterTC significantly decreased than these in 24h. Furthermore, pre-test failures (PTF: described in [PTF] in the table) were confirmed in these products excluding SACR-01.

Conclusions SBS of current self-adhesive composite resin products was significantly decreased with observation of PTF by thermal cycling load. However, SACR-01 showed the highest bond strength to both enamel and dentin among tested products and there was no significant change on SBS by thermal cycling load. It may be expecting that SACR-01 can bring good clinical results.

Table 1 SBS of self-adhesive composite resin products

<table>
<thead>
<tr>
<th>SBS/MPa (S.D.)/[PTF]</th>
<th>Enamel 24h</th>
<th>Enamel afterTC</th>
<th>Dentin 24h</th>
<th>Dentin afterTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SACR-01 (GC)</td>
<td>16.7 (4.7) A</td>
<td>18.9 (0.8) A</td>
<td>15.1 (2.0) a</td>
<td>12.7 (3.3) a</td>
</tr>
<tr>
<td>FIT SA (SHOFU)</td>
<td>18.0 (6.9) A</td>
<td>12.0 (7.1) [1] AB</td>
<td>12.1 (7.1) a</td>
<td>0 (0) [5] b</td>
</tr>
<tr>
<td>Fusio (Pentron)</td>
<td>10.8 (2.0) ABC</td>
<td>0.6 (1.3) [4] D</td>
<td>9.1 (2.6) ab</td>
<td>0 (0) [5] b</td>
</tr>
<tr>
<td>Vertise Flow (Kerr)</td>
<td>11.6 (1.7) ABC</td>
<td>1.2 (2.7) [4] CD</td>
<td>9.4 (2.0) ab</td>
<td>1.8 (4.1) [4] b</td>
</tr>
<tr>
<td>Constic (DMG)</td>
<td>14.7 (0.7) A</td>
<td>4.3 (4.3) [2] BC</td>
<td>7.0 (7.4) ab</td>
<td>0 (0) [5] b</td>
</tr>
</tbody>
</table>

Tukey: Enamel : Upper case letter, Dentin : Lower case letter
**P305**

**Bond Strength of Glass-Hybrid and Glass-Ionomer Materials to Primary Dentine**

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**Objectives** Although new formulations of glass-ionomer (GI) restorative materials have been introduced, little is known about their performance in primary dentition. The aim of the study was to evaluate the microtensile bond strength (µTBS) of a glass-hybrid (GH), high-viscous (HV-) and resin-modified (RM-) GI restoratives to sound (SD) and caries-affected (CAD) primary dentine.

**Methods** Occlusal cavities were prepared in 60 primary molars and randomly divided into two groups: SD and CAD. Teeth (n=6) were restored with a GH (Equia Forte HT, GC Int- EF), two HV-GI (Equia Fill, GC Int- E; Ketac Molar, 3M ESPE- KM), and two RM-GI (Fuji II LC, GC Int- II, Photac Fill, 3M ESPE- PF) materials. Samples were immersed in artificial saliva at 37°C for 7 days, and subsequently exposed to the thermal aging (10.000×). Twelve microspecimens per group were formed and allowed to age in artificial saliva at 37°C for 7 days. µTBS was tested at loading rate of 0.5 mm/min until failure. Mode of failure was analyzed using an optical digital microscope (magnification 300×).

**Results** The overall bond strength was higher for SD than for CAD (p<0.05, Mann-Whitney test). However, when µTBS to SD and CAD was compared for individual materials, difference was significant for E (5.07 MPa, 2.41 MPa), and PF (2.57 MPa, 0.93 MPa), while EF (4.05 MPa, 3.73 MPa), II (3.27 MPa, 1.93 MPa) and KM (1.40 MPa, 0.79 MPa) showed no differences. For both SD and CAD, EF and E showed higher µTBS compared to other GI restoratives (p<0.05, Kruskal-Wallis test). The most frequent mode of failure was adhesive, followed by mixed failure, with no differences between the subgroups (p>0.05, chi-square test).

**Conclusions** Novel restorative formulations are likely to provide better bonding properties to both sound and caries-affected primary dentine compared to the previous generations of GI materials.

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**P306**

**Bonding a Universal Adhesive to Silver Diamine Fluoride Treated Dentin**

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**Objectives** To determine whether the ability of a mild universal adhesive bonded in self-etch mode to sound dentin would be affected by the use of silver diamine fluoride and potassium iodide following different bonding protocols.

**Methods** Exposed flat-mid-coronal dentin surfaces were polished with 320-grit silicon carbide paper. Crown segments were randomly divided into 6 groups (n=5 teeth/group) according to the use of 38 % silver diamine fluoride (Riva Star, SDI; SDF), silver diamine fluoride and potassium iodide (Riva Star, SDI; SDF+KI), or no treatment (Control) followed by application of a mild universal adhesive (Scotchbond Universal Plus Adhesive, 3M-ESPE) under self-etch mode to sound dentin. µTBS to SD and CAD was compared for individual materials, difference was significant for EF (5.07 MPa, 2.41 MPa), II (3.27 MPa, 1.93 MPa) and KM (1.40 MPa, 0.79 MPa) for immediate bonding, significantly higher bond strengths to air-abraded SDF or SDF+KI treated dentin were obtained. After 7 days, all bonding protocols produced equivalent bond strengths compared to untreated dentin (p>0.05).

**Conclusions** Silver diamine application had deleterious effects on dentin bond performance of a mild universal adhesive. Air abrasion can restore bond strengths of SDF treated dentin, however a delayed bonding of at least 7 days are needed to revert bond strength loss produced by SDF+KI.
P308
Bonding of Composite to Fiber-Reinforced Resin – Effect of Fiber Orientation
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Objectives Fiber-reinforced composite (FRC) is promoted as a digitally-fabricated alternative for the fabrication of fixed restorations. A composite-veneering is mandatory for FRC. In this study, short- and long-term shear bond strength between FRC and veneering composite were evaluated. The effect of fiber sheet orientation with respect to the bonding surface was also of interest.

Methods By vertical sectioning, CAD/CAM-FRC blanks (Trinia, Bicon LLC) were divided into blocks. These blocks where cut to discs with 2mm thickness using angulations of either 2° (n=104) or 45° (n=102) with respect to the horizontal plane. The final sample cross-sections measured 10mm x 10mm. After fixation in acrylic resin the samples were ground planar. As recommended by the manufacturer, the samples were sandblasted before primer, bonder and opaquer layers were applied (Cera-Resin Bond and Universal Opaque, Shofu Dental). A cylindrical pin (diameter: 2.38mm) of light-curing veneering-composite (Ceramage, Shofu Dental) was added to the sample surface in layering-technique. Half of the specimens of each group were subjected to either short-term water storage (24h at 37°C) or long-term water storage (180d). Shear bond strength tests were conducted as described in ISO29022 in a universal testing device (2005, Zwick/Roell). The surfaces of the cut FRC discs possessed a layered structure (alternating glass fiber and epoxy resin layers). For half of the specimens of each subgroup the shear force was either in parallel or perpendicular with respect to the layer orientation.

Results Compared to samples tested after 1d (23.9MPa), mean shear bond strength values were significantly lower after long-term water storage (18.1MPa, p<0.001) but still above the 10-MPa-threshold often used for clinical recommendation. The cutting- (2°=21.4MPa, 45°=20.6MPa, p>0.584) and loading- direction (perpendicular=21.6MPa, parallel= 20.4MPa, p=0.367) had no significant effect.

Conclusions Based on the results, veneering of FRC can be recommended for clinical use, irrespective of the fiber sheet orientation.

P309
Short-Term and Aged Dentin-Bonding Effectiveness of Self-Cured Universal Composite Cements
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Objectives This study aimed to measure the bonding effectiveness of two universal composite cements to bur-cut dentin using a shear bond-strength (SBS) and micro-tensile bond-strength (µTBS) approach. All cements, and primers/adhesives when used, were applied in solely self-cure mode as the most challenging luting condition.

Methods Short-term SBS was measured 10 min upon luting to P600-grit SiC-paper ground dentin, representing a clinically relevant surface condition for semi/indirect luting procedures. One-week and 6-month aged µTBS was measured to 46-µm diamond bur-cut dentin, again simulating semi/indirect luting conditions. The universal composite cements G-CEM One with/without Adhesive Enhancing Primer (‘Gone_AEP’ and ‘Gone’, GC) and RelyX Universal with/without Scotchbond Universal Plus (‘RU_SBUp’ and ‘RU’, 3M Oral Care) were compared to Panavia V5 applied with Tooth Primer (‘Pv5_TP’, Kuraray Noritake) serving as reference, totalling to 5 experimental groups (n=10). Data were statistically analyzed using 1- and 2-way Anova, respectively. Fractographic analysis was carried out by light microscopy and SEM.

Results The highest 10-min SBS was recorded for Pv5_TP, being significantly higher than that of Gone_AEP, RU and RU_SBUp. Regarding µTBS, Gone_AEP performed statistically equally well as Pv5_TP upon 6-month aging, while the 1-week µTBS of Pv5_TP was still significantly higher than that of Gone_AEP. Significantly lower 1-week/6-month µTBSs were recorded for Gone, RU and RU_SBUp without any mutually significant difference except for RU, revealing a significantly lower µTBS than RU_SBUp upon 6-month aging.

Conclusions The touch-cure polymerization mechanism used by the composite cements G-CEM One (GC), when combined with Adhesive Enhancing Primer (‘Gone_AEP’), and Panavia V5 (Kuraray Noritake), when combined with Tooth Primer (‘Pv5_TP’), improved the short-term and aged dentin-bonding effectiveness of the composite cements applied in self-cure luting mode.
Bond Strength of Fiber Posts: Universal Versus Self-Adhesive Resin Cements

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Objectives To determine and compare the push-out bond strength of six resin cements used to lute fiber post along the radicular length

Methods Thirty roots received endodontic treatment and were randomly divided into six experimental groups according to the resin cement used to lute the fiber posts (FRC Postec Plus size 0 and 1) into the root canal: three self-adhesive resin cements (RelyX Unicem, RX; G-Cem, GC; NormoCem, NC), two universal resin cements (RelyX Universal, RU; G-Cem One, GO) and one self-etch resin cement used as control (Multilink Automix, MA). Resin cements were light-cured through the post (Spec3) according to manufacturers instructions. After 24 h moisture storage, six slices were transversally obtained from each root, two corresponding to each root third: cervical, middle, and apical thirds. Then, push-out test was performed at a crosshead speed of 0.5 mm/min using a universal testing machine (Instron 3345) and bond strength was expressed in MPa. Type of failure was analyzed under a stereomicroscope (Olympus SZX7). Data were analyzed by two-way ANOVA and Fisher’s exact test (p<0.05).

Results Mean values and standard deviations obtained were as follows for each resin cement: RX (12±7.2), GC (12.7±5.6), NC (13.4±7), RU (13±4.5), GO (13±7), MA (13.8±5). The variables resin cement (p=0.86), root third (p=0.19) and the interaction between both factors (p=0.05) did not influence on bond strength results. The type of failure observed was affected by the resin cement used (p=0.045).

Conclusions Similar bond strength results were obtained with universal and self-adhesive resin cements tested, irrespectively the root third. The most prevalent type of failure found was adhesive for all cements, except for the control group MA for which mixed and adhesive failures were equally observed.

Comparison of Different Universal Adhesive Systems on Dentin Bond Strength

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Objectives To compare the shear bond strength of composite resin to dentin using new universal adhesives after different types of storage.

Methods Specimens were prepared from caries-free third molars using a low-speed saw, embedded in an acrylic resin and wet ground to create a flat bonding area. Three bonding agents; G2-Bond Universal (GC), Clearfil SE Bond (Kuraray) and Scotchbond Universal Plus (3M ESPE) were applied to the dentin. Bulk-fill composite cylinders were created by filling teflon mold with SDR flow+ (Dentsply Sirona). Samples were randomly divided into subgroups (n=10) and subjected to different aging times (two-months incubation or two months incubation and thermal-cycling) and adhesive procedures (total-etch or self-etch). Thermal-cycling process was four days in temperature 55°C and 5°C, that corresponds to one year of physiological aging in the oral cavity. Shear bond strength was evaluated by loading the specimens in bond strength testing machine UltraTester (Ultradent). Bond strength data (MPa) were analyzed using two-way ANOVA and Tukey’s test.

Results Results revealed that shear bond strength was significantly affected by the thermal-cycling (P=0.018) and different adhesive systems use (P=0.001). Scotchbond Universal Plus had lowest MPa values (average values with and without thermal-cycling) and is significantly different from G2-Bond Universal (regardless of adhesive mode). MPa values of Clearfil SE Bond are between there two groups and no significant differences were observed compared to them.

Conclusions Our findings indicated that the new adhesive G2-Bond Universal has desirable shear bond strength to dentin compared to other universal adhesives, even after one year in the oral environment.
Universal Adhesives Silane Content Influences Bond Strength to Glass-Ceramic
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Objectives This study aimed to evaluate the effect of γ-methacryloxypropyltrimethoxysilane (γMPTS)-containing and γ-methacryloxypropyltriethoxysilane (γMPTES)/3-(aminopropyl)triethoxysilane (APTES)-containing universal adhesives (UAs) on resin–ceramic microtensile bond strength (µTBS).

Methods Lithium-disilicate ceramic (LDC) (GC Initial LiSi Block, GC) discs were acid-etched with 5% hydrofluoric acid, cleaned and randomly distributed into four groups (n=10) according to the priming material utilized. In group 1 (control), the LDC discs were not primed; group 2, a universal primer (Monobond N (MBN), Ivoclar Vivadent) was applied; group 3, γMPTS-containing UA (Single Bond Universal Adhesive (SBU), 3M Oral Care) was used as a primer and group 4, γMPTES/APTES-containing UA (Scotchbond Universal Plus Adhesive (SBP), 3M Oral Care) was used as a primer. LDC discs were cemented using a dual-cure resin cement (RelyX Universal Resin Cement, 3M Oral Care), then sectioned into micro-beams (1mm × 1mm) for µTBS evaluation and tested after 24h water storage using a universal testing machine. Failure modes were assessed. µTBS data were analyzed using one-way analysis of variance (ANOVA) and Tukey’s multiple comparison tests (α = 0.05).

Results Universal primer (MBN) application showed the highest µTBS (32.21 ± 4.49 MPa) among all groups (p<0.05). γMPTES/APTES-containing UA (SBP) resulted in significantly higher µTBS (20.41 ± 6.26 MPa) compared to γMPTS-containing UA (SBU) (10.65 ± 3.5 MPa) or the control group (8.47 ± 3.11 MPa) (p < 0.05). There was no statistically significant difference between γMPTS-containing UA (SBU) and the control group (p > 0.05). Mixed and adhesive failure modes were the most predominant in all groups.

Conclusions Silane content incorporated in UAs can influence resin–ceramic µTBS. While γMPTES/APTES-containing UA improved bonding to LDC, priming of LDC with either of the UAs tested cannot be considered as an alternative to a separate silanization (priming) step using a universal primer.
Bonding Hydrophobic-Rich Resins to Demineralized-Dry Dentin Using DMSO

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**Objectives** To evaluate the effect of a dimethyl sulfoxide (DMSO) pretreatment, following different application times, and drying protocols on resin-dentin bonding performance of methacrylate-based monomer combinations.

**Methods** Mid-coronal dentin surfaces from sound molars were etched with H3PO4 for 15 s and randomly divided into 7 groups. Etched surfaces were blast-dried, pretreated with a 50% (v/v) DMSO ethanolic solution (20 or 60 s) or not pretreated and further air-dried for 30 s. For each pretreatment condition, bonding was performed using the conventional primer-and-bond combination (P+B) or employing only the hydrophobic-rich bond component (B) of an etch-and-rinse adhesive (Scotchbond Multi-Purpose, 3M ESPE). Wet-bonded samples using (P+B) served as a control. Bonded teeth (n=6/group) were stored in distilled water (37 °C/24 h) and sectioned into resin-dentin beams (0.9 mm2) for microtensile bond strength testing (0.5 mm/min). Fracture patterns were characterized under SEM. Bond strengths were analyzed with ANOVA and Tukey’s test (α=0.05).

**Results** Bond strength effectiveness depended on DMSO application time and resin combination. Untreated-dry samples produced significantly lower bond strengths than DMSO-treated samples (p<0.05). While 60 s DMSO-dry bonding with P+B produced significantly higher bond strengths than the conventional P+B wet bonding (p<0.05), bonding with only B produced equivalent bond strengths (p>0.05). 20 s DMSO-dry bonding with P+B produced equivalent bond strengths to P+B wet bonding (p>0.05) and bonding with only B produced lower values (p<0.05).

**Conclusions** Although conventional bonding approaches failed to directly bond hydrophobic-rich resins to demineralized-dry dentin, DMSO/EtOH pretreatments can circumvent such limitations under appropriate application times. Moreover, DMSO may reduce the technique sensitivity of dry bonding within clinical acceptable times. DMSO-dry bonding may contribute to improved resin-dentin interface durability and reduce bonding dependency on hydrophilic-rich monomeric primers.

In Vitro Response of Human Dental Pulp Stem Cells to Dental Composite.

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**Objectives** Dental composite resins represent some of the most used materials in modern dentistry. Composite materials can exert diverse effects on different target tissues. Monomers could inhibit specific cellular functions of odontoblasts or influence the odontogenic differentiation and mineralization processes in pulp-derived cells, including stem cells. The aim of this preliminary study was to evaluate cytotoxicity of two novel composite and whether they can affect mineralization of human dental pulp stem cells (hDPSCs).

**Methods** Ceram.x SpectraTM (Dentsply Sirona), a nanohybrid composite with pre-polymerized Sphere-Tec particles, and Admira Fusion (Voco), a nanohybrid restorative material with the innovative technology ORMOCER were tested. The samples and the eluates were prepared according to ISO 10993-12:2012. We treated hDPSCs in undifferentiated state with eluates for 4 days and performed MTT assay to evaluate cytotoxicity of dental composites. The effect of the eluates on the hDPSCs mineralization capacity was assessed after osteogenic differentiation by alizarin red staining.

**Results** We tested different dilutions of both eluates and observed that undiluted Ceram.x eluates were significantly cytotoxic over time, reducing cell viability of about 51.7% as compared untreated cells. On the other hand, the eluates obtained from ORMOCER technology composite did not display any adverse effect on hDPSCs viability. Moreover, we assessed composites effects on ostogenesis, demonstrating that Ceram.x limited osteogenic activity of hDPSCs, while Admira Fusion did not appear to show any adverse effect.

**Conclusions** Our data show that composite with conventional dental monomer might exert cytotoxicity on hDPSCs as compared to ORMOCER technology. Indeed, the limited cell differentiation caused by Ceram.x could be explained by its the cytotoxic effect. In this regard, the study is continuing to define cellular changes induced by the composites, and to further identify the optimal dental composite resin for clinical use.
Impact of Colour-Vision Deficits on Shade Selection Among Dental Students

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Objectives To evaluate the prevalence of colour-vision deficits among a sample of Portuguese final year dental students, and to correlate these findings with their accuracy in shade selection using a commercial tooth shade scale.

Methods A total of 119 final year dental students from Instituto Universitário Egas Moniz (IUEM, Caparica, Portugal) were recruited. Participants were mainly female (75%) and had an age interval of 22-42 years old, with a mean age of 23. The evaluation comprised two stages: an initial colour-vision screening test, based on the Ishihara test, and a posterior corresponding shade selection test using the VITAPAN classical scale (Vita Zahnfabrik, Germany). The Ishihara test was applied under standardizes lighting conditions and included reading numbers displayed inside colored circles. For the second phase of the study, students were given 3 teeth from the VITAPAN classical scale and had to blind match the tooth shade with a complete VITAPAN scale. Descriptive statistics were employed using SPSS v. 26.0.

Results Out of the studied sample, only males presented colour-vision deficiency (4/119 wrongly identified all discs from the Ishihara test). Regarding the VITAPAN classical scale match, in total, 59.4% of the students gave the correct answer. The male gender obtained 54.8% of the correct correspondences, while females presented 61%, with no difference reported between genders. Students found it easier to correctly matched shades within the following order of matrices, C>B>A>D. On average, darker shades were more difficult to be correctly matched than lighter ones.

Conclusions The presence of colour vision deficiencies does not seem to affect the correct identification and choice of tooth shade using a commercial scale. The male gender showed higher prevalence of colour-vision deficiencies. Considering the tooth shade scale, easiness of matching seems dependent upon the matrix.

Efficacy of Three Different Bleaching Techniques – Randomized Controlled Trial

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Objectives Evaluate the effect of different bleaching techniques in tooth color by visual and spectrophotometric methods.

Methods A randomized controlled trial was designed with three parallel groups (n=30 per group): Group A – in-office 6% hydrogen peroxide (HP) paint-on varnish; Group B – at-home 6% HP with adaptable tray; Group C – at-home 16% carbamide peroxide (CP) with custom tray. At three different stages (baseline, post-treatment, and six-month follow-up) the buccal surfaces’ VITA Classical shades of the upper canines and central incisors were determined by visual method (with a calibrated operator) and by a spectrophotometer (with an independent operator). The acquired shades were converted to CIE L*a*b* values by previously described methods and used to assess tooth color difference ($\Delta E_{00}$). Reliability analysis was performed at all stages with the intraclass correlation coefficient (ICC) between both tooth color assessment methods. Results were presented as mean and 95% confidence intervals and statistical tests were performed appropriately, considering a significance level of $\alpha$=0.05. It was considered the perceptibility/acceptability threshold (PT/AT) of $\Delta E_{00}$=0.8/1.8.

Results The ICC ranged from poor to good reliability, yet it decreased in every group post-treatment. In all bleaching groups both visual and spectrophotometric methods detected $\Delta E_{00}$ values higher than the AT at post-treatment and higher than the PT at six-month follow-up in the canine teeth (compared to post-treatment). Most visual’s post-treatment detected color differences (visual $\Delta E_{00}$ canine/incisive: Group A - 4.5[4.1:5.0]/3.5[2.9:4.1]; Group B - 4.1[3.8:4.4]/2.8[2.3:3.4]; Group C - 6.7[6.2:7.3]/3.0[2.5:3.4]) were significantly (P<0.05) higher than the spectrophotometer (spectrophotometer $\Delta E_{00}$ canine/incisive: Group A - 3.5[3.1:3.8]/2.2[1.5:2.9]; Group B - 3.4[3.0:3.9]/2.0[1.3:2.7]; Group C - 6.5[5.9:7.1]/2.6[2.0:3.2])

Conclusions Bleaching efficacy and tooth color relapse were detected in all techniques with both assessment methods. However, higher tooth color differences were detected by the visual method which presented a lower reliability for post-treatment analysis.
P316

Color Change of Universal Composites After Polishing

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Objectives To determine the effect of polishing on the color of previously stained universal composites by immersion in different beverages.

Methods A total of 125 circular specimens (10 mm diameter and 2 mm thickness) of four universal resins (Filtek Supreme XTE, Essentia Universal, Omnichroma, Ceram.X Spectra ST, Filtek Universal and a multishade one Filtek Supreme XTE. Specimen surfaces were polished with 2500 and 4000 grit SiC discs. After 48 hours of immersion in distilled water, the samples were randomly distributed and immersed in 30 ml of the following beverages (30 ml) (n=5) for 3 months at 37°C: distilled water, coffee, Cola and red wine. Afterwards, samples were polished using a spiral polishing system (EVE Diacomp Plus TWIST). CIELab color coordinates were measured by a spectrophotometer (VITA Easyshade) initially, after 3 months of immersion and after polishing. Color differences (ΔE) were calculated and data were analyzed by two-way ANOVA, Tukey’s test and paired t-tests (p<0.05).

Results After 3 months, ΔE values were influenced by the composite, the beverage tested and the interaction between both factors (p<0.001). Omnichroma exhibited the lowest color change after immersion in coffee and red wine, without differences with Ceram.X Spectra ST in the latest beverage. After polishing, ΔE values decreased in the wine- and coffee-immersed samples and did not in those immersed in Cola and water.

Conclusions Polishing partially recovered the original color of universal resin composites previously immersed in wine and coffee, although the ΔE values remained were clinically relevant for most of the composites.

P317

Color Stability of One-Shade Resin Composites After Immersion in Beverages

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Objectives To determine the color stability of one-shade resin composites after 1-month immersion in colored beverages.

Methods 60 specimens (10 mm diameter and 2 mm thickness) of two one-shade resin composites, Omnichroma (Tokuyama) and Unique (FGM), and a multishade one, Filtek Supreme XTE (3M Oral Care) were prepared and subsequently polished with 2500 and 4000 grit SiC discs. After 48 hours of immersion in distilled water, the samples (n=5) were randomly distributed and immersed for 1 month at 37°C in the following beverages (30 ml): distilled water, coffee, Cola and red wine. Afterwards CIELab color coordinates were measured by a spectrophotometer (VITA Easyshade) under a gray background and the parameter ΔE was calculated. Results were analyzed by two-way ANOVA and Tukey’s tests (p<0.05).

Results After 1 month, ΔE values were influenced by the composite, the beverage tested and the interaction between both factors (p<0.001). Unique composite did not exhibited a relevant color change after immersion in water or Cola, unlike Omnichroma. However, immersion in coffee and red wine produced a significant increase of ΔE for the resin composites tested. The highest ΔE values were determined for Filtek Supreme XTE after immersion in red wine.

Conclusions Red wine and coffee produced a significant color change for all resin composites tested. The color of one-shade composite Unique was not affected by immersion in Cola.
Translucency and Value of CAD/CAM Glass-Ceramic Veneers After Hydrothermal Aging

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Objectives To evaluate the translucency parameter (TP) and value (L) change of different glass-ceramic CAD/CAM blocks in two different thicknesses (0.5mm, 1mm) after hydrothermal aging.

Methods Four types of CAD/CAM blocks; lithium disilicate (IPS e.max CAD, Ivoclar Vivadent; E.max, leucite-reinforced (Initial LRF, GC; LRF), zirconia-reinforced lithium silicate (Celtra Duo, Dentsply; CD), and feldspathic (Cerec, Dentsply; CER) glass-ceramics were cut in 0.5mm and 1mm (n=12) using a low-speed diamond saw (Isomet). To represent dental hard tissues, a total of 96 resin-composite discs (G-eneial, GC; A2, 2mm) were prepared. Then CAD/CAM slabs and resin composite substrates were luted with light-cured resin cement (G-CEM; GC) and polymerized (VALO, Ultradent) according to the manufacturer’s instructions. Immediate after cementation, initial measurements (TP0, L0) were evaluated using a spectrophotometer (CM-2600d, Minolta). Following storage in distilled water (24h, 37°C), second measurements (TP1, L1) were performed. Then all the specimens were exposed to hydrothermal aging (5-55°C) for 30000 cycles (SALUBRIS). Measurements were repeated after every 10000 cycles. TP and L values were evaluated by CIEDE2000. Data were analyzed with three-way ANOVA and post hoc Tukey’s tests (p<0.05).

Results TP and L values were significantly affected by ceramic type (p<0.001), ceramic thickness (p<0.001), and aging (p<0.001). Regarding ceramic thickness, all tested materials had significantly different TP values between 0.5mm and 1.0mm (p=0.001). With respect to ceramic type, in 0.5mm CER presented significantly lower TP than LRF, CD, and E.max (p=0.019, p<0.001, p<0.001, respectively). CER 0.5mm (p=0.045) and CD 1.0mm (p=0.028) resulted in significantly lower TP after 30000 cycles. All ceramic materials exhibited significantly lower L values after aging (p<0.001).

Conclusions Ceramic type, thickness, and aging affected translucency and value parameters of glass-ceramics. Translucency change of the feldspathic glass-ceramics was thickness-dependent, while regardless of thickness, the value of all tested glass-ceramics decreased after aging.

Sixty-Month Follow-up of a Glass Hybrid in NCCLs

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Objectives To evaluate the clinical performance of a glass hybrid (GH) compared with a nano ceramic composite resin (CR) in non-carious cervical lesions (NCCLs) of patients with bruxism after 60 month.

Methods Twenty-five patients with NCCLs and bruxism were enrolled in this study. The dimensions of the NCCLs (depth, cervico-incisal height, and mesio-distal width), internal angles, degree of tooth wear (TWI) and gingival conditions (GI) were recorded. A total of 148 NCCLs were randomly restored with a GH (Equia Forte Fil, GC, Tokyo, Japan) or a nano-ceramic CR (Ceram.X One Universal, Dentsply, DeTrey, Konstanz, Germany). The restorations were evaluated at baseline and after 12, 24, 36 and 60 months according to the modified USPHS criteria. Statistical analyses were performed using chi-square, McNemar’s and Kaplan Meier tests. The level of significance was set at p < 0.05.

Results At the 60-month recall, 97 restorations in 15 patients were evaluated. The recall rate was 60.0%. Survival rates were 73.5% for CR and 66.7% for GH. A total of 29 restorations were lost [13 CR (26.5%), 16 GH (33.3%)]. No significant difference was found between the restorative materials for retention (p=0.232), marginal adaptation (p=0.935) and marginal discoloration (p=0.643). No relationships were found between internal angle, depth, cervico-incisal height or mesio-distal width and retention of the GH or CR restorations (p>0.05). The increase in retention loss and marginal discoloration of both restorations over time were found significant (p<0.001). Both restorations showed no secondary caries or tooth sensitivity at any evaluation time.

Conclusions GH and nano-ceramic CR showed similar clinical performance and survival. The use of GH proved to be an alternative restorative material in the restoration of NCCLs of patients with bruxism during the 60-month follow-up.
Gingival Displacement Systems Efficacy Before Impression: Multicenter Randomized Clinical Trial

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Objectives Quantitative assessment of gingival sulcus width (lateral displacement) obtained by three retraction systems (Racestyptine + Cord, Racegel + Cord and Racegel) in order to collect clinical performance and safety data from them (Retract study).

Methods Design of the trial was prospective, multi-center randomized, controlled, open label, 3-arm parallel group. The primary endpoint was the lateral gingival displacement.

In this post-market clinical follow up study, both Racestyptine Solution and Racegel are investigational devices. No device was used as comparator, each gingival retraction system was compared to a reference value of minimal expected tissue displacement.

Results The lateral gingival displacement obtained by Racestyptine with a retraction cord (253 ± 59 µm, p<0.0001) and by Racegel with a retraction cord (247 ± 61 µm, p=0.0007) were significantly higher than 200 µm. Lateral displacement obtained with Racegel cordless was 207 ± 57 µm (p=0.53). Astringent effect of the three retractions systems was confirmed by the absence of crevicular fluid reported on more than 96% of the patients and by bleeding observed on 7 cases. No peri-odontal damage was observed immediately and 7 days after retraction. Prosthesis fits correctly on the prepared tooth for all patients of the 3 groups.

Conclusions RETRACT Study shows that Racestyptine and Racegel provides a sufficient sulcus opening prior impression taking in restorative dentistry.
P323

The Importance of Dental Cement in Determining Dental age

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Objectives The aim of the study was to assess the correlation between cellular cement thickness and age and in male and female subjects and on teeth with preserved crown and with large carious destruction.

Methods The study sample consisted of 57 donor teeth of both sexes. The teeth used were extracted because of orthodontic or prosthetic reasons. Donors’ age ranged from 10 to 70 years at the time of tooth extraction. Each tooth was cut with transverse incisions on an ISOMET 1000 cutter in the apical, middle and cervical third of the tooth’s root. Cement thickness measurements were done using a light microscope and Olympus EP50 camera, Version: V3_20190202. The correlation between cement thickness and chronological age was calculated using the Spearman correlation coefficient. Random picked 20% of cuts were measured by two researchers to estimate the inter-rater agreement.

Results A positive correlation was found between cementum thickness and known chronological age of the donor (r = 0.47, p<0.001). The effect of independent variables (sex, number of tooth roots, and condition of the tooth crown) on the estimated thickness of the cellular cementum and was greater on the cervical part. Perfect agreement was found (mean whole root cement thickness, apical and middle third, kappa> 0.86; cervical third cement thickness, kappa = 0.815) for inter-rater agreement, yielding a very small measurement error of 0.89% (95% CI -0.21 up to 1.98%).

Conclusions The thickness of the cellular cementum was greater in men, especially in the cervical third of the tooth root with a destructed crown. The study shows that the obtained quantitative values of cellular cement thickness can be a reliable tool used in estimating the dental age of humans.
Evaluating Buccal and Occlusal Polished Enamel Surfaces Following Erosion.
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Objectives Tooth wear is a multifactorial condition with chemical, biological and behavioral factors involved in the etiology and pathogenesis. The majority of in-vitro studies to assess the impact of various erosive solutions have been conducted using buccal polished enamel surfaces. It is not known whether the buccal or occlusal surface is more susceptible to erosion and if so it might explain clinical observations. The aim of this study was to compare step height loss on polished buccal and occlusal surfaces following erosion.

Methods Enamel specimens (n=20) were cut from the coronal aspect of previously extracted human molars. The samples were divided into two groups, buccal and occlusal (n=10); all the specimens were polished to achieve a flatness tolerance of ±0.9µm, confirmed by surface profilometry. Adhesive tape, applied to the surface, was used to create a window of exposed enamel and two protected zones of reference in 1:3 ratios. Both groups were treated with 0.3% citric acid at pH 2.7 and fully immersed and stirred at 62rpm for 5, 10, 15, 20, 40, or 60 mins. The mean step height was measured using confocal non-contact white light laser profilometry.

Results The mean surface loss measured in step height (um) and standard deviation (SD) at 5, 10, 15, 20, 40 and 60 mins for the buccal and occlusal surfaces were 3.17(0.58), 5.61(0.75), 7.83(0.97), 10.89(1.71), 19.99(1.51), 31.26(3.64), and 3.63(0.65), 6.35(0.86), 8.76(1.28),11.63(1.43), 20.85(1.92), 32.92(3.43) respectively. There was no significant difference between the two areas following erosion.

Conclusions On extracted human teeth and following erosion, buccal and occlusal polished surfaces had similar susceptibility to erosion.

Angiogenic and Osteogenic Potentials of an Injectable Lysine Dendrigraft Hydrogel
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Objectives Due to their tunable mechanical properties and biocompatibility, poly(ethylene glycol) (PEG) hydrogels have been widely used as scaffolds for tissue engineering applications. A drawback of these hydrogels is their bio-inert nature which can modified by grafting dendrimer-linked functionalized molecules (dendrigrafts). This strategy allows a selective adhesion of cells to induce target tissue regeneration. The aim of this study was to investigate the angiogenic and osteogenic potential of a new poly(L-lysine) dendrigrafts (DGL)/PEG-based hydrogel for bone regeneration.

Methods PEG and DGL were emulsified into a hydrophobic solution (surfactant and oil) to form microspheres. The hydrogel toxicity was evaluated on Human Umbilical Vein Endothelial Cells (HUVECs) and Mesenchymal Stem Cells (MSCs) by the MTT test. To investigate the angiogenic potential, hydrogel microspheres were cultured with HUVECs. After 24h, HUVECs organization into tube-like structures was evaluated using optical microscopy and the supernatants were harvested to quantify TGF-β1 growth factor secretion by ELISA. To investigate the osteogenic potential, after incubating HUVECs with the microspheres, MSCs migration towards HUVEC supernatants was investigated using Boyden chambers. Finally, MSCs were cultured with microspheres to check their osteogenic differentiation by evaluating Alkaline Phosphatase activity (ALP) and quantifying BMP-2 by ELISA.

Results The functionalized hydrogel microspheres were not toxic to HUVECs nor to MSCs. The direct contact between the microspheres and HUVECs induced a significant increase of TGF-β1 secretion and a tube-like organization. Incubation of HUVECs with the hydrogel microspheres significantly induced MSCs recruitment. Moreover, incubating MSCs with the hydrogel microspheres significantly enhanced their ALP activity and BMP-2 secretion.

Conclusions This poly(L-lysine) dendrigraft (DGL)/PEG-based hydrogel enhanced the angiogenic and osteogenic potentials and represents a promising tool in tissue engineering for bone regeneration.
Comparison of Cell Detachment and Counting Procedures of Osteoblasts Cultured on Titanium Surfaces

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Objectives In vitro studies of osteoblast responses to dental implant materials may provide a useful indication of potential bone healing around the implant. Osteoblast viability and cell numbers are often assessed on implant surfaces. The reliability of cell counts following enzymatic detachment is unclear when dealing with confluent mineralised matrix forming cultures and in situ nuclear staining presents difficulties on opaque surfaces. This study compared two cell detachment and two counting methods from titanium surfaces often used as a dental implant material.

Methods 15000 osteoblast-like human Saos-2 cells were incubated on titanium surfaces in osteogenic culture media containing $10^{-7}$ M dexamethasone, 2 mg/ml β-glycerophosphate and 0.05 mg/ml ascorbic acid for 7 days to allow the production of mineralised extracellular matrix. Cell cultures without the osteogenic supplementation and/or incubated on polystyrene surfaces served as controls. Viable cells (trypan blue exclusion) were counted using a haemocytometer or an automatic cell counter (Spark, Tecan, Switzerland), following detachment using a 10 min incubation with either 0.05% trypsin alone or in combination with 0.8% collagenase-I. Any remaining cells on the Ti surface were observed with scanning electron microscopy (SEM) following a standard preparation procedure.

Results Following osteogenic supplementation, cell detachment with trypsin alone revealed lower viable cell numbers than with trypsin and collagenase-I. This difference was not observed in control cultures. SEM of osteogenically treated cultures showed cells remaining on the Ti indicating incomplete cell detachment following enzymatic treatment. Counting with an haemocytometer and trypan blue staining produced less variable, more robust results when compared with the automated cell counter ($9.6 \times 10^4 \pm 0.2 \times 10^4$ and $10.2 \times 10^4 \pm 40.2 \times 10^4$ cells respectively).

Conclusions Collagenase-I in combination with trypsin improved the detachment of osteogenically supplemented osteoblasts from titanium surfaces compared with cells detached using trypsin alone, despite incomplete cell detachment.

Structure of Human Tooth Enamel Tuft Revealed by Microtomography

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Objectives The aim of our study is to reveal 3D structure of the human dental tufts. The tufts are structures localized at the enamel-dentin junction and whose origin is not clear. Previous studies have shown that the tufts are hypomineralized and contain more protein compare to enamel. This organization could explain long term resilience of teeth.

Methods Ten adult mandibular first and second molars and two decidual mandibular first molars were cut. Then, sample were scanned with a resolution microcomputed tomography. A resolution of around 5 micrometers were obtained. Then 3D meshes of surface of DEJ, include the 3D tuft pattern, were reconstructed.

Results The tufts are well organized structure in adult teeth selected, with a length which could reach to 1 mm. Regular undulations are clearly visible, with 30 degrees orientation from junction and spatial frequency of approximately 160 micrometers.

Conclusions Enamel human tuft have a draped pattern, maybe connect to orientation of group of prisms. The undulation could be related to other structure seen in teeth: Hunter-Schreger bands. More powerful tool, as synchrotron X-ray microtomography, is necessary for deciduous tooth.
Impact of Passive Ultrasonic Irrigation on Radicular Dentin.

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Objectives To analyze the composition of radicular dentin by ATR-FTIR after the use of different concentrations of NaOCl in combination with 17% EDTA, with and without passive ultrasonic Irrigation (PUI).

Methods A sample of each root third from 16 single-rooted human teeth was selected. Specimens were randomly distributed according to the irrigation procedure into: (1) 2.25% NaOCl (20 min) +17% EDTA (1 min) +2.25% NaOCl (1 min); (2) 5.25% NaOCl (20 min) +17% EDTA (1 min) +2.25% NaOCl (1 min); (3) 2.25% NaOCl (19 min) +2.25% NaOCl with PUI (1 min) +17% EDTA with PUI (1 min) +2.25% NaOCl with PUI (1 min); (4) 5.25% NaOCl (19 min) +5.25% NaOCl with PUI (1 min) +17% EDTA with PUI (1 min) +5.25% NaOCl with PUI (1 min). Carbonate/mineral, Amide I/mineral and Amide III/CH2 ratios were measured before and after the treatments. Results were analyzed by U Mann-Whitney test (p<0.05).

Results The carbonate/mineral ratio significantly increased after irrigation with 2.25% NaOCl with PUI meanwhile decreased for 5.25% NaOCl concentration, with or without PUI. The Amide I/mineral ratio was not affected by the activation when the concentration of NaOCl was 2.25%. The Amide III/CH2 ratio significantly increased after activation with PUI of 2.25% NaOCl concentration. Without PUI, 2.25% NaOCl produced significantly lower Amide I/mineral and Amide III/CH2 ratio values than 5.25% NaOCl. The 5.25% NaOCl with PUI procedure significantly decreased both Amide I/mineral and Amide III/CH2 ratios.

Conclusions The combined treatment of NaOCl and EDTA activated by PUI, showed changes in the organic and inorganic composition of radicular dentin. The most remarkable change was for 5.25% NaOCl concentration activated with PUI.

Minimal Intervention Dentistry for Children With Caries - a Feasibility Study

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Objectives Dental caries presents a significant public health problem. Minimal Intervention Dentistry (MID) comprising early detection, diagnosis, prevention, restoration, and recall is an approach to managing dental caries in children. To test if MID in treating caries in children’s permanent teeth is more effective than a conventional approach, a randomized control trial (RCT) is required. However, several uncertainties exist about the feasibility of running an RCT in primary care including MID complex intervention delivery. This study aimed to investigate these uncertainties.

Methods Dental professionals at selected dental practices were invited to participate. They received training on MID and were asked to deliver a course of MID to children with caries in their permanent teeth. A mixed methods approach was used to collect data. Stakeholders including dentists, dental care professionals, children, and their parents were asked to complete questionnaires with a sample invited to take part in semi-structured interviews exploring their views on participation. These were audio-recorded, transcribed verbatim, and analysed using framework analysis.

Results Primary care dental practices (n=10) were selected with all located in areas in the most deprived 50% of areas in England. The training workshops were delivered: dentists (n=31), dental care professionals (n=13). After training 18 dental professionals delivered MID intervention to children (n=81) who had carious lesions that ranged from early to advanced (ICDAS codes 1 to 6). Themes including perceived value of research participation, MID intervention delivery, recruiting patients, and team involvement were identified.

Conclusions Dental professionals thought that delivering an MID intervention to children was acceptable and were supportive of a future definitive trial. Children and parents were also accepting of MID. Further analysis is needed to cover additional parameters of uncertainty before a decision can be made on a definitive trial.
A Novel TGF-β3-Derived Chondroinductive Peptide to Promote Chondrogenesis

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Objectives The chondral repair of large cartilage defects remains highly challenging in the fields of orthopedics and oral and maxillofacial surgery due to inadequate chondrogenic microenvironment. One viable approach is to adopt chondroinductive growth factors, such as BMP-2 and TGF-β3. However, their use is associated with various limitations, such as low production yield, high cost, and potential immunogenicity. To provide a viable alternative, in this study, we wished to develop a novel chondroinductive peptide and its-functionalized hydrogel to repair large cartilage defects.

Methods We analyzed the crystallographic data of the critical binding domain of TGF-β3 with its type II receptor and designed 10 TGF-β3-derived peptides (TPs). In an ectopic (intramuscular) cartilage induction model in rats, we found that TP8 (adsorbed onto absorbable collagen sponge) potently induced de novo cartilage formation. Thereafter, we analyzed the efficacies of TP8 in inducing in-vitro chondrogenesis of BMSCs in a micromass chondrogenesis model. We also explored the molecular mechanisms for the chondroinductivity of using western blot and RNA sequencing. At last, we histomorphometrically evaluated the in-vivo efficacy of TP8-functionalized Gel-MA hydrogels to repair the cartilage defects in rabbit medial femoral condyles using the International Cartilage Repair Society (ICRS) visual histological scoring evaluation system.

Results 500 ng/ml TP8 induced a significantly higher area of glycosaminoglycans in the micromass of BMSCs than 500 ng/ml BMP-2 or 10 ng/ml TGF-β3. Moreover, TP8-functionalized Gel-MA hydrogels were associated with significantly higher ICRS visual histological scoring evaluation system than the un-functionalized ones. Western blot analyses showed that TP8 significantly upregulated phosphorylated Smad1/5. Thereafter, RNA sequencing analyses showed that 356 mRNAs were upregulated and 104 mRNAs were downregulated in the TP8-treated mouse BMSCs in comparison with the untreated mouse BMSCs. We found that differentially expressed mRNAs are associated with the MAPK and PI3K-Akt signaling pathways by KEGG enrichment analysis. Our next step was to explore differentially up-regulated genes in the relevant mRNAs.

Conclusions These data suggested a promising application potential of TP8 in repairing large cartilage defects.
P334

Measuring Quality-of-Life After Customized TMD-Treatment; What Affects It Most?
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Objectives Investigate oral-health-related-quality-of-life (OHRQoL), and pain after attending customized TMD-treatment with multidisciplinary approach, and look further into what factors affects OHRQoL among TMD-patients.

Methods A cross-sectional study was conducted to investigate TMD-patients OHRQoL after attending customized TMD-treatment. A questionnaire was distributed to former TMD-patients of Oral Health Center of Expertise Rogaland, Norway. The questionnaire contained questions regarding their OHRQoL (OHIP-14), their current pain levels and treatment experience. Background factors, psychosocial and physical baseline variables (PHQ-4, GCPS2.0, JFLS-8) measured pre-treatment were retrieved from their dental journal. A principal component analysis was run to investigate the psychometric properties of OHIP-14. To further investigate correlation between OHIP-14 and questionnaire data, background factors, psychosocial and physical variables, non-parametric correlation analysis was conducted.

Results Response rate was 32.5% (n=65), 84.6% were female. 84.4% of respondents were satisfied/very satisfied with the treatment. The average OHIP-14 sum score was 16.2(SD=10.8). A principal component analysis revealed two factors representing psychosocial limitations and discomfort, and physical pain. The treatment did not significantly reduce self-reported pain, however the difference in pain level was positively correlated with OHIP-14. Further non-parametric correlation analysis showed positive correlation between OHIP-14 sum score and number of health-professionals involved during the treatment, chronicity, physical pain, psychological distress, functional and psychosocial limitation, and oral parafunctional behavior. Interestingly, OHIP-14 showed no correlation with the self-management, but there was positive correlation between treatment satisfaction and the self-management.

Conclusions This study indicates that OHRQoL among TMD-patients are poorer than the general population even after treatment where majority of the respondents were satisfied/very satisfied with the overall treatment. Furthermore, OHIP-14 in this population shows moderate to strong correlation between physical and psychosocial variables as well as number of health-professionals involved accentuating the need for a multidisciplinary approach in TMD management.

P337

Anti-Fungal Effects of CMFs on MRONJ and Diabetic Foot
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Objectives The aim of this study was to evaluate the anti-fungal and the tissue regenerative effects of Complex Magnetic Fields (CMFs) (MFI, Rome, Italy) on patients with Medication-related Osteonecrosis of the Jaws (MRONJ) and diabetic foot.

Methods Previous research from our Laboratory has shown, in in vitro studies, an anti fungal effect of CMFs. MRONJ and Diabetic foot are two forms of necrotic tissue areas that have different pathogenesis but similar local complications due to the formation of an aggressive bacterial biofilm, composed also by candida. Healing times of three cases of MRONJ and three cases of Diabetic foot were compared. After debridement of the necrotic areas with a mini-invasive treatment based on necrotic tissues removal and coverage with platelet hemoconcentrate, CMFs were used, with an appropriate external device. The application times were 1 hour per day for a total of 45 days.

Results The results showed a complete healing of the necrotic areas.

Conclusions These preliminary results have shown that the anti-fungal effects of CMF could be an important support to treat necrotic infected areas in MRONJ and in diabetic foot. Further studies, with a higher number of patients, will be necessary to confirm these results.
Impact of COVID-19 on Head and Neck Cancer Presentation
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Methods All patients diagnosed with Head and Neck Cancer in Northern Ireland are managed through a Multi-disciplinary Network based in the Royal Victoria Hospital in Belfast. Patient level data was collected for all patients who were managed through this network during 2019, 2020 and 2021. In addition to demographic information, specific data on cancer diagnosis, tumour site, tumour staging and treatment provided were collected prospectively.

Results The total number of patients referred to the Multi-disciplinary Network in 2019 was 470 however this decreased to 371 in 2020 (22.1% reduction, p<0.01). During this same period the proportion of late stage tumours (T3 / T4) increased from 33.76% of all cases in 2019 to 41.47% (p=0.015) in 2020. In 2021, the data suggests a return to similar patterns recorded in 2019 with 436 cases managed in total, of which 36.70% were late stage tumours. The treatments offered to patients during the period 2019-2021 remained broadly similar with Surgery (34.1%) the most common modality followed by Radiotherapy (29.68%). It was noted that in 2021, a higher proportion of patients received Radiotherapy (30.5%) compared to Surgery (29.59%) with an increased proportion referred for specialist palliative care. The data also revealed that the number of confirmed cases referred urgently (‘red flag referrals’) by General Dental Practitioners decreased significantly in 2020 and 2021 (p<0.01) compared to 2019.

Conclusions This data suggests that the COVID-19 pandemic had significant negative impacts on Head and Neck Cancer presentation in Northern Ireland. The total number of cases referred to the Multi-disciplinary Network decreased significantly in 2020 with a higher proportion presenting as late stage tumours.

P351
Antimicrobial CHX-CaCl2 Particle Coatings for use on Maxillofacial Fixation Devices.
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Objectives To synthesise CHX-CaCl2 particles on to maxillofacial fixation devices and characterise the drug release and antimicrobial efficacy.

Methods Buttons (Grp. 1) were coated with 1ml chlorhexidine (15 mg/ml CHX), followed by 1ml of CaCl2 (0.3 M) and left for 1 min. As a commercial comparison, Leonard Buttons (Grp 2) were pipetted with 2 ml of 0.2% Corsodyl for 1 min. The coated samples surfaces were characterized using SEM. Grp. 1 and 2 samples (n=3 per group) were transferred to tubes of artificial saliva (AS) and CHX release was measured at specific time points using UV-Vis Spectroscopy. At each time point the media was changed. The aliquots from selected time points (24, 48, 72 and 144h) were used to measure antibacterial efficacy against S. mutans and P. gingivalis strains. The aliquots were inoculated into 96-well microtiter plates containing equal amounts of broth media, and bacterial suspension (0.1 OD). The plate was then placed in the plate reader for analysis at absorbance 600 nm then re-measured after 24h incubation for bacterial evaluation. X-Ray Diffraction was used to analyse the crystallinity of CHX-CaCl2, compared to commercial CHX and CaCl2.

Results XRD analysis revealed a unique crystal structure for the CHX-CaCl2 particles. SEM displayed CHX-CaCl2 crystal coating of a dendritic nature on Grp. 1 specimens, and no visible coating on Grp. 2 specimens. Grp. 1 illustrated a sustained drug release of up to 3 days compared to Grp. 2 of up to 40 mins. The aliquots from Grp. 1 inhibited S. mutans (<144h) and P. gingivalis strains at all time points measured.

Conclusions Successful coating of novel CHX-CaCl2 particles on to Leonard Buttons was displayed and a sustained drug release compared to the commercial comparison. This is useful for future applications within Oral and Maxillofacial surgeries in lowering localized infections.
How did Containment Measures Affect Dental Prescribing Patterns During COVID-19?
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Objectives Starting in 2020 pandemic of Covid-19 has made an impact on everyday clinical work and consequently medication prescribing for dental practitioners. During a 2-year time, measures have been modified, depending on each country's government decisions. In Croatia in 2020 stringency index was strong, while in 2021 index was very weak and in spring 2022 restriction measures were suspended. We investigated the impact of the pandemic and restriction measures on the dental prescribing pattern.

Methods Data related to prescription practice were delivered by the Croatian Health Insurance Institute for the years of 2019. - 2021. The number of dentists’ prescriptions, the cost of medicines, and the number of packages prescribed have been included in the analysis.

Results Changes in prescription patterns that could be attributed to the restriction's measures were seen in the results of this study in analgesic and antiseptic prescriptions. For the most of medications from these groups, the rise was great only while the measures were strict in the first year, while in the second year of the pandemic the increase significantly dropped. An exception from this behavior is seen for the ibuprofen, whose utilization is showing a continuous increase for the observed time period as well as for the 5 previous years. The most prescribed medications were antibiotics with no significant changes in their prescribing pattern for the pandemic period. Wide spectrum antibiotics utilization is showing a slow but continuous increase while narrow spectrum is in decrease. Pandemic has made an impact only on prescribing of azithromycin, whose utilization increased between years for 39,4%, and 8,7% respectively. The reason for this anomaly is to be investigated.

Conclusions Restricted access to dental care due to COVID-19 resulted in changes to the prescription pattern of dental medications. The changes that could be attributed to the restriction measures are seen in pain relief medications, antiseptics, and wide-spectrum antibiotic azithromycin. Adaptation to the Covid-19 pandemic setting in dentistry is now over, and observed abnormalities have to be corrected primarily through evidence-based dental prescribing protocols and guidelines, in order to ensure rationality in medication prescribing and good clinical practice.
P342
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Objectives Evaluation of the appropriate and optimum amount of the support structures for 3-dimensional (3D) printing processes for denture base fabrication is required when the support structure is a critical part that affects the surface quality of the final products and impacts the environment by its wastefulness while retaining the accuracy. This study aims to analyse the trueness and eco-efficiency when reducing the support structure to minimize material consumption, printing time, cost, and environmental impaction.

Methods A maxillary complete denture base construction file has been used as a reference. Four different support structure conditions were used to 3D printed twenty denture bases (total n=80): no support structure reduction (Control), palatal support structure reduction (Condition P), border support structure reduction (Condition B), and palatal and border support structure reduction (Condition PB). For trueness, scans of the printed dentures were superimposed onto the original reference file to evaluate the geometric accuracy further using nonparametric Kruskal-Wallis and Steel-Dwass tests (α=0.05) assess the denture accuracy. The outcome values from general data were used to assess the environmental and economic performances. An eco-efficiency analysis was employed to integrate the evaluated findings of the environmental and economic performances into a single framework to aid decision-making by selecting the optimum and most eco-efficient alternative.

Results While there was no significant difference in trueness, Condition PB provides the most appropriate behaviors in environmental and economic performances—an overall material savings of around 16%, considering the complete parts, by 18% of energy consumption (kWh) and carbon emission (kg CO₂) savings, with 13% cost-effective in economic compared to the Control. Therefore, Condition PB was revealed as the most eco-efficient.

Conclusions Condition PB is suggested due to its proper support structure distribution and retaining an acceptable accuracy range with the most eco-efficient.

P343
Comparison of Algorithm-Based Occlusion Method With Classical Occlusion Alignment Method
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Objectives This study compared the alignment of digital jaws performed by two different methods of occlusal analysis: one based on a bite scan and the other based on a specific algorithm (Intercusp, Rechenraum GmbH), known as Smart Occlusion, which saves times as a bite scan is not needed. The aim is to investigate if the Smart Occlusion method results in reliable and accurate occlusion like the bite scan method. The first hypothesis is that the contact zones between upper and lower jaws are the same for both methods. The second hypothesis is that Smart Occlusion method will have no intersections between upper and lower jaw, since this is mathematically not possible in this method.

Methods A total of 20 sets of upper and lower jaw were scanned (ALS II, GC Europe NV) and aligned first with the bite scan and next with the Smart Occlusion. GOM Inspect was used to calculate the distances between upper and lower jaws for each set and visualize the occlusal contact areas (distances ≤ 0.5 mm) for both methods. The total intersecting area (area with distances < 0) was also calculated.

Results All 20 cases showed a reliable occlusion and both methods generated similar occlusion contact areas. None of the Smart Occlusion alignments (0%) had intersecting upper and lower jaws. In contrast, 16 of the 20 scan-based alignments (80%) had intersecting upper and lower jaws. The average intersecting area over those 16 cases was 3.6 square mm.

Conclusions Smart Occlusion is a timesaving, efficient and reliable method to align digital jaws. It provides the same occlusion contact areas as the classical scan-based method, but without intersections between upper and lower jaws. Smart Occlusion reduces the number of alignments with intersecting jaws by 80%.
**Evaluation of the Shape Accuracy of Pre-Sintered Co-Cr Alloy Clasps**

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**Objectives** The development of pre-sintered Co-Cr alloy discs has added a new option to the fabrication of removable partial dentures (RPDs). Crown fabricated from pre-sintered Co-Cr alloy discs have been reported to fit well, there have been no reports on clasps for RPDs. The purpose of this study was to compare the shape accuracy of Co-Cr clasps fabricated under three conditions: casting, milling from fully-sintered alloy discs, and milling from pre-sintered alloy discs.

**Methods** After fabrication of wax pattern for Akers clasp on refractory cast, a 3D scanning was performed and clasp data was obtained using CAD software (design data). Clasps were fabricated under the following conditions. Cast clasp: fabricated by casting technique. Fully-sintered clasp: fabricated by milling from fully-sintered discs based on design data. Pre-sintered clasp: fabricated by milling from pre-sintered discs based on design data. The fabricated clasps were 3D scanned, and the clasp data were obtained (fabrication data). The design and fabrication data were superimposed to calculate the shape accuracy. Statistical analyses were performed using Kruskal-Wallis test and Steel-Dwass tests (P<0.05).

**Results** Cast clasp showed a higher trueness than the other two conditions, and statistically significant differences were observed among the three conditions. Fully-sintered clasp showed better precision than the other two conditions, and statistically significant differences were found among the three conditions. The results suggest that sintering shrinkage influence the trueness of clasp tip for pre-sintered clasp. Fully-sintered clasp showed excellent precision, but insufficient cutting was observed at the base of the rest. Pre-sintered Co-Cr alloy discs has the advantage of milling at a larger size than the design data before sintering, which allows for more detailed milling, suggesting the possibility of superior accuracy.

**Conclusions** The results suggest that pre-sintered clasp is outstanding in term of fabrication accuracy if the sintering shrinkage could be controlled.

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**Differents Direction on Precision of Temporary Bridges Using 3D Printers**

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**Objectives** The aim of this study was to investigate the effect of constructive direction (layer orientation) on dimensional changes in a provisional partial multiple dental restoration on SLA, DLP, LCD and 4 k printers.

**Methods** Forty (80) temporary fixed partial restorations were printed with a hybrid composite resin. Four impression groups were formed, in addition to four groups according to the construction process (layer orientation) of said restorations.

**Results** Mix and Phrozen systems in vertical orientation, where the opposite was observed: SLA<sub>H</sub>=0.03 mm; SLA<sub>V</sub>=0.06 mm; DLP<sub>H</sub>=0.11 mm; DLP<sub>V</sub>=0.16 mm; PHR<sub>H</sub>=0.09 mm; PHR<sub>V</sub>=0.06 mm; WM<sub>H</sub>=0.05 mm; WM<sub>V</sub>=0.06 mm. Regarding the contrast between the systems regarding printing errors, only the differences between DLP and Whip Mix were significant (p=0.03), while the differences according to orientation were not significant (p=0.64). Regarding the accuracy of the systems, in general terms the most accurate printing system was SLA, followed by DLP, Whip Mix and finally Phrozen.

**Conclusions** Impression accuracy was acceptable in all systems, although the accuracy of the systems revealed a degree of uncertainty that could be considerable from a clinical point of view. Somewhat better results were achieved with the SLA system.
**Digital Evaluation of the Fit of Screw-Retained Implant-Supported Prostheses**  
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**Objectives** To evaluate the fit of two-implant-supported screw-retained zirconia prostheses on malpositioned implants by performing digital screw resistance test.  

**Methods** Thirty-two-implant-supported screw-retained zirconia prostheses were fabricated and divided into 3 groups (n=10) according to different abutment combinations: both engaging (E-E), engaging and nonengaging (E-NE), both nonengaging (NE-NE). The fit of each prosthesis was tested on the control cast and on 6 definitive casts simulating 50-, 100-, and 150-µm vertical and 35-, 70-, 100-µm horizontal implant positioning errors. The abutment screws were tightened on each implant and the screw rotation angle was measured digitally with a custom-made digital ratchet and a computer software program. The accuracy of the digital ratchet was validated by angle and torque measurements (n=10) on several occasions. The data were statistically analyzed by one-way ANOVA and the Tukey’s test (p<0.05).  

**Results** E-E specimens on the 100-µm horizontal error group and on all vertical error groups were clearly ill-fitting and were excluded. Statistically significant differences among groups with different combinations of the abutments were found (p<0.05). The engaging abutments had higher angle of rotation compared with the nonengaging abutments on all casts. In the horizontal error group, E-E specimens had the highest angle of rotation, followed by E-NE and NE-NE specimens. In the vertical error group, the E-NE specimens had the highest angle of rotation on the side of the engaging abutment. Angle of rotation increased with increasing level of error.  

**Conclusions** With an increased level of the simulated implant positioning error, the angle of rotation increases. NE-NE specimens were found to tolerate best the different error levels followed by E-NE and E-E combinations. Horizontal errors caused higher angle of rotation than vertical ones, and could therefore be regarded as more detrimental.  

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**Accuracy of Pure Titanium RPD Frameworks Using Various CAD/CAM Techniques**  
Kosei Ito, Akinori Tasaka, Hiro Kobayashi, Seiichi Nakata, Shuichiro Yamashita  
1Department of Removable Partial Prosthodontics, Tokyo Dental College, Chiyoda, Tokyo, Japan, 2Wada Precision Dental Laboratories Co., Ltd., Osaka, Japan  

**Objectives** Commercially pure titanium (CP-Ti) is a lightweight and biocompatible material, but its difficulty in casting technique has been a major problem in fabricating removable partial denture (RPD) framework. In recent year, it has become possible to fabricate the frameworks using CAD/CAM techniques. However, the shape accuracy of the frameworks is unknown. The purpose of this study was to clarify the influence of fabrication techniques on the shape accuracy of CP-Ti RPD framework using CAD/CAM technology.  

**Methods** Kennedy class II partially edentulous mandibular model was used for the simulation model. After scanning of the model was performed using a dental scanner, the framework was designed by using CAD software (design data). The following four techniques were used to fabricate the RPD framework: (1) Ti-milling: milling from CP-Ti disc, (2) Ti-resin: casting technique from CP-Ti ingot using 3D printed resin pattern, (3) Ti-wax: casting from CP-Ti ingot using milling wax patterns, and (4) Ti-SLS: selective laser sintering from CP-Ti powder. Ten frameworks were fabricated for each CAD/CAM technique. 3D scanning of fabricated 4 types of frameworks were performed (fabrication data), and these data were overlapped with design data. The shape accuracy among CAD/CAM techniques were compared using the Steel-Dwass test after Kruskal-Wallis test (α=0.05).  

**Results** The median trueness was 0.05 mm with Ti-milling, 0.03 mm with Ti-resin, -0.01 mm with Ti-wax, and 0.00 mm with Ti-SLS. The median precision was 0.02 mm with Ti-milling, 0.05 mm with Ti-resin, 0.07 mm with Ti-wax, and 0.01 mm with Ti-SLS. There was a statistically significant difference in the trueness and precision among CAD/CAM techniques for most of the measurement sites.  

**Conclusions** The results suggest that CAD/CAM techniques were found to influence the shape accuracy of CP-Ti RPD framework.
Clinical Outcomes of 3D-Printed Bridges on Immediately Loaded Implants
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Objectives Patients’ expectations for replacing missing teeth are becoming more demanding, and some refuse to be partially edentulous even for a short period of time. Treating both full and partial tooth loss with an immediate, fixed restoration will have a positive effect on quality of life. This “case-series” aimed to look at same-day loading of fully tapered titanium implants restored with a temporary two or three-unit bridge. The presented material is part of a 10-year prospective study where implant survival after immediate loading is the primary aim.

Methods Sixteen bridges, supported by two implants, were installed in 15 patients within the time frame defined as “immediate loading”. The patients went through intraoral scanning immediately after implant placement, and 3D-printed bridges were manufactured in acrylic-resin material. The bridges were screwed on to the implants within hours after surgery. The patients were followed-up, one, four and eight weeks after the surgical procedure. Radiographs, clinical photographs, and clinical examinations of the patients were carried out at each visit. The resin-bridges were replaced with permanent prosthetic restorations after three months. Technical complications with the temporary prosthetic restorations were registered. As was biological complications as implant loss, signs of inflammation and patients’ subjective complaints of pain or discomfort.

Results Within the three months use, one temporary bridge fractured upon tightening of the prosthetic screw on the implant abutment, and one prosthetic screw loosened. One implant was lost. No other biological complications were recorded for the implants.

Conclusions Within the limits of this study, in particular the small sample size, 3D-printed resin bridges can be considered a reliable solution as temporary prosthetic restoration for immediate loading of fully tapered titanium implants.

Enamel Demineralization Caused by Various Carbohydrates Challenges - in Vitro Evaluation
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Objectives Creating a simple reproducible in vitro dental caries model of demineralization is a challenge. Simplifying multiple factor in vitro caries models including carbohydrate challenges, bacteria and dental products are needed.

Methods Fifty-seven enamel slabs (4x4mm) cut from human extracted teeth were used. A single species S. mutans 72-hour biofilm was created over the enamel surfaces. The specimens were then exposed to three carbohydrate challenges (10% sucrose, Keyes #2000 or brain–heart infusion (BHI)), 3 times a day for 15 minutes over the course of four days. pH was measured after each exposure. After four days, viable counts (CFU/mL) and biofilm biomass (crystal violet staining) were evaluated. Demineralization was evaluated clinically and by Vickers microhardness test. Slabs were photographed using Nikon SMZ25 stereomicroscope before and after exposure to caries promoting conditions.

Results The BHI carbohydrate challenge measured the least amount of enamel demineralization. Compared clinically to 10% sucrose and Keyes #2000 carbohydrate challenges, these enamel slabs were shinier, and healthier with a less deminerlized chalky enamel. Biofilm biomass tests presented identical bacterial growth between the three groups. Viable counts on the other hand were very different, BHI (~1010), 10% sucrose (~109) and Keyes #2000 (~107).

Conclusions The results demonstrate that the 10% sucrose carbohydrate challenge has the most reliable and reproducible behavior and should be the favored protocol for in vitro caries model. Each carbohydrate challenge produces a different environment and effect on bacterial viability.
The Effect of Coca-Cola Zero© and S. Mutans on Enamel Demineralization- in Vitro Evaluation

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Objectives Sugary drinks such as coca cola© may expedite dental caries. Consequently, people diverted to consume sugar free drinks like coca cola zero©. This research aims to evaluate in-vitro the coca cola zero© effect in the presence of S. mutans biofilm on enamel demineralization.

Methods Sixty-nine human enamel slabs (4x4mm) were used. S. mutans UA-159 72-hour biofilm was created over enamel surfaces. The specimens were then exposed to coca cola zero©, HCL and 10% sucrose, 3 times a day for 15 minutes over the course of four days. pH was measured after each exposure. After four days, viable counts (CFU/mL) and biofilm biomass (Crystal Violet staining) were evaluated. Demineralization was evaluated clinically and by Vickers microhardness tests. Slabs were photographed using Nikon SMZ25 stereomicroscope before and after exposure to caries promoting conditions.

Results Slabs exposed to coca cola zero© showed an increase in viable counts of up to 2 logs compared with 10% sucrose exposures (10^{10} Vs. 10^8 ) Coca cola zero© pH measures were the lowest and the only group to show a decrease in pH over time (pH~3). Biofilm biomass tests showed a 25% higher bacterial growth in the coca cola zero© group. Enamel slabs that were evaluated clinically in the stereomicroscope post exposures had a chalky and matt appearance as opposed to their shiny appearance in the baseline evaluation.

Conclusions Coca cola zero© creates a favorable environment for the growth of S. mutans. This implies that even though coca cola zero© is sugar free it has a cariogenic effect on the enamel slabs.

Oral Squamous Cell Carcinoma Diagnosis From Salivary Amino Acid Profile

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Objectives To find out the association of salivary amino acid levels with oral squamous cell carcinoma (OSCC).

Methods This case-control study was conducted from December 2020 to February 2022. Patients with histologically confirmed OSCC were enrolled as cases in the study from Oral and Maxillofacial Surgery Department, Bangabandhu Sheikh Mujib Medical University (BSMMU) and Dhaka Dental College Hospital (DDCH), Mirpur 14, Dhaka by convenient sampling. Total 55 samples were taken where 34 were OSCC patients and 21 were healthy controls. Saliva was taken from both group and ensuring proper environment was carried to Bangladesh Reference Institute for Chemical Measurements (BRiCM), Science lab, Dhanmondi for analysis by Liquid Chromatography-Mass Spectrometry (LC-MS). By this machine, the levels of 6 specific amino acids (Proline, Valine, Phenylalanine, Isoleucine, Leucine, and Lysine) were measured. The data obtained from each subjects was recorded and was analyzed using the SPSS version 24 and level of significance was considered \( p<0.05 \). Ethical approval was taken from all institutions.

Results Majority of the subjects in cases 23(67.6%) were aged between 51 -70 years and in controls 11(52.4%) were aged between 31-50 years. Proline and Leucine levels among the cases were significantly higher and Lysine value of the cases was significantly lower than the healthy controls. Multivariate logistic regression showed that oral habitual factors, leucine and lysine level were significantly associated risk factors for OSCC.

Conclusions Salivary proline, leucine and lysine concentration showed significant difference between OSCC patients and healthy subjects in this study. This suggests that, salivary amino acid profile might be helpful in early, effective, non-invasive and rapid diagnosis of OSCC.
Distribution of the study subjects by educational status (n=55)

Age distribution of the study subjects

<table>
<thead>
<tr>
<th>Age group in years</th>
<th>Case (n=34)</th>
<th>Control (n=21)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-30</td>
<td>0(0)</td>
<td>1(4.8)</td>
<td>0.150</td>
</tr>
<tr>
<td>31-50</td>
<td>11(32.4)</td>
<td>11(52.4)</td>
<td></td>
</tr>
<tr>
<td>51-70</td>
<td>23(67.6)</td>
<td>9(42.8)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34(100)</td>
<td>21(100)</td>
<td></td>
</tr>
</tbody>
</table>

P-value was determined by Chi-square test. Table shows that majority 23 (67.6%) of the patients in cases were aged between 51-70 years and in controls 11 (52.4%) were 31-50 years. But no significant age difference found between two groups.

Salivary amino acid levels among the study subjects

<table>
<thead>
<tr>
<th>Salivary amino acid levels (µmol/L)</th>
<th>Case (n=34)</th>
<th>Control (n=21)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proline</td>
<td>43.9±23.7</td>
<td>21.5±23</td>
<td>0.001</td>
</tr>
<tr>
<td>Leucine</td>
<td>10.8±16.2</td>
<td>3.2±4.7</td>
<td>0.044</td>
</tr>
<tr>
<td>Valine</td>
<td>12.9±22</td>
<td>9.7±4.8</td>
<td>0.510</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>13.6±14.7</td>
<td>10.7±7.8</td>
<td>0.410</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>9.5±17.7</td>
<td>3.0±3.0</td>
<td>0.105</td>
</tr>
<tr>
<td>Lysine</td>
<td>9±7.9</td>
<td>16.6±8.9</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*P-value was determined by Independent sample t test. P< 0.05 was considered significant. Table shows that Proline and Leucine level among the cases were significantly higher than the controls. Besides, Lysine value of the cases was significantly lower than the healthy controls.

Risk factors analysis of oral squamous cell carcinoma by Multivariate logistic regression (n=55)

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Education (below SSC)</td>
<td>1.414</td>
<td>0.136-14.687</td>
<td>0.772</td>
</tr>
<tr>
<td>Oral habitual factors</td>
<td>91.829</td>
<td>2.192-3846.731</td>
<td>0.018</td>
</tr>
<tr>
<td>Poor oral hygiene</td>
<td>2.152</td>
<td>0.248-18.674</td>
<td>0.487</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------</td>
<td>--------------</td>
<td>-------</td>
</tr>
<tr>
<td>Proline</td>
<td>0.966</td>
<td>0.916-1.019</td>
<td>0.200</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.706</td>
<td>0.540-0.922</td>
<td>0.011</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.228</td>
<td>1.054-1.432</td>
<td>0.009</td>
</tr>
</tbody>
</table>

P-value was determined by Multivariate logistic regression. By multivariate logistic regression this table shows that oral habitual factors, leucine level and lysine level were significantly associated risk factors for oral squamous cell carcinoma.