Workshop for

Nordic Young Scientists in

Oral Research

2 - 4 September 2019

Oslo

Norway

Programme at a glance

MONDAY, SEPTEMBER 2nd	
13:00 - 14:00	Lunch
14:00 - 14:15	Opening and welcome ceremony
14:15 - 15:00	**Session 1**: Dr. Kerstin Galler, *Dental pulp - repair and regeneration*
15:00 - 16:00	Students presentations
16:00 - 16:30	Coffee break
16:30 - 17:15	**Session 2**: Dr. Roger Simm, *Biofilms and antibiotic resistance: the curse of modern medicine*
17:30 - 18:30	Poster session
19:15 - 20:00	Get together/live music
20:00	Dinner

TUESDAY, SEPTEMBER 3rd	
09:15 - 10:00	**Session 3**: Dr. Andreas Stavropoulos, *Translating periodontal regeneration*
10:00 - 11:00	Students presentations
11:00 - 11:30	Coffee break
11:30 - 12:30	Students presentations
12:35 - 13:00	Sponsor presentation: NOF
13:00 - 14:00	Lunch
14:00 - 17:00	Social activity and walk
17:00 - 17:45	**Session 4**: Dr. Bente Brokstad Herlofson, *Jaw bone pathology – a challenge for the wave of new medical treatments in cancer and osteoporosis*
17:45 - 19:00	Poster session and sponsors' showcase
19:00	Dinner

WEDNESDAY, SEPTEMBER 4th	
09:15 - 10:00	**Session 5**: Dr. Kamal Mustafa, *From bench to chairside, stem cells in bone augmentation*
10:00 - 11:00	Students presentations
11:00 - 11:15	Coffee break
11:15 - 12:00	Awards and closing ceremony

Sponsors

UiO : Life Science
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Abstracts for keynote speakers

1. Professor Kerstin Galler

Department of Conservative Dentistry and Periodontology, University Hospital Regensburg, Germany

DENTAL PULP – REPAIR AND REGENERATION

To maintain pulp vitality is an essential goal in dentistry. With advances in the fields of pulp biology and regenerative medicine, more biology-based treatment approaches are considered today. In the case of reversible pulpitis or traumatic pulp exposure, vital pulp treatment is indicated and shows satisfactory success rates. In the case of pulp necrosis, new treatment protocols exist for teeth with incomplete root formation, where revitalization is a viable alternative to an MTA plug. With this approach, repair can be induced, but not true regeneration, thus future perspectives for dental pulp regeneration based on tissue engineering strategies will be discussed.

2. Associate Professor Roger Simm

Institute of Oral Biology, University of Oslo, Norway

BIOFILMS AND ANTIBIOTIC RESISTANCE: THE CURSE OF MODERN MEDICINE

Antimicrobial resistance is an increasing problem worldwide and one of the most important threats to public health. Biofilms are inherently resistant to antimicrobials, and can provide environments for fast development and dissemination of antimicrobial resistance. This complicates therapeutic intervention of biofilm mediated diseases and threatens the future of modern medicine. An increasing number of persistent infections are recognized to be biofilm mediated. This includes common oral diseases such as caries, chronic periodontitis and persistent endodontic infections. The current understanding of biofilm related antimicrobial resistance, the challenges associated with treatment of biofilm infections and potential novel therapeutic options will be dicussed, with a focus on oral diseases.

3. Professor Andreas Stavropoulos

Department of Periodontology, Faculty of Odontology, Malmö University, Sweden

TRANSLATING PERIODONTAL REGENERATION

Deep pockets after periodontal therapy, especially in sites/teeth harboring deep intrabony defects, are associated with an increased risk for disease progression and tooth loss. Various treatment protocols have been thus during the years aiming to enhance clinical treatment outcomes, but also with the intention to enhance periodontal regeneration. Periodontal regeneration implies, that CAL gain is achieved through new cementum with functionally oriented inserting collagen fibers formed on the previously exposed/affected portion of the root, paralleled with new alveolar bone formation and the establishment of a periodontal ligament of physiologic width and composition. Several technologies have been used during the years aiming at periodontal regeneration, including broad use of bone substitute materials. In general, both the clinical and histological outcomes obtained after such approaches have been significantly better compared to conventional surgical approaches, but it is also relevant that these improved outcomes can be maintained on the long-term. Can advances in tissue engineering further enhance the outcomes?

4. Professor Bente Brokstad Herlofson

Department of Oral Surgery and Oral Medicine, Institute of Clinical Dentistry, University of Oslo, Norway

JAW BONE PATHOLOGY – A CHALLENGE FOR THE WAVE OF NEW MEDICAL TREATMENTS IN CANCER AND OSTEOPOROSIS

With the expected increase in the aging population, the dental society will need to address and manage more complex patients with comorbidity and comedication. Diseases and their treatments may affect patients` oral health and quality of life. One in three will become cancer patients. In Norway more than 250 000 patients are suffering from osteoporosis. New treatment strategies in cancer and osteoporosis care are inundating the health care system. People live longer with their diseases and oral complications are expected to increase in the years to come. There is a constant need for knowledge on oral health care related side effects of new therapies. The presentation will include information on the ongoing umbrella project “Oral morbidity in cancer and cancer treatment” with a specific focus on jaw pathology related to medical treatment. The Scandinavian osteonecrosis of the jaw research collaboration during 8 years on medication-related osteonecrosis of the Jaw in cancer and osteoporosis patients in Sweden, Denmark and Norway will be presented.

5. Professor Kamal Mustafa

Department of Clinical Dentistry, University of Bergen, Norway

FROM BENCH TO CHAIRSIDE, STEM CELLS IN BONE AUGMENTATION

The use of advanced therapy, in the form of cell therapy and tissue engineered products, has been recently increased and considered as a promising new approach in regenerative medicine.

Substantial amount of work and efforts have been placed aiming to develop a novel synthetic bone substitute that triggers bone healing in patients and used as a carrier for mesenchymal stem cells (MSC). Our recent clinical data showed that the microporous beta-calcium phosphate (MBCP) granules induce the formation of new bone and could be combined with autologous MSC for reconstruction of atrophied alveolar bone.

Based on the evidence from preclinical and clinical studies, the safety and regenerative potential of autologous MSC in combination with scaffolds have been demonstrated. Furthermore, preclinical data underscore the importance of 3D-printed scaffolds for the successful outcome in bone regeneration.

Abstracts for students presentations

1. Oral and poster presentations

____ Presenting author

OP 1 BIOACTIVE GLASS AIR-ABRASION ENHANCES WETTABILITY AND OSTEOBLAST PROLIFERATION ON SANDBLASTED AND ACID-ETCHED TITANIUM SURFACES

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BACKGROUND: Sandblasting and acid-etching are commonly used surface modifications for oral implants. However, the same modification can promote biofilm formation once the surface is exposed to the oral environment. This may eventually lead to peri-implantitis and implant failure. The ideal management of peri-implant diseases focuses on decontamination of implant surfaces and regeneration of lost tissues.

OBJECTIVES: This study aimed to evaluate the hydrophilicity and surface free energy of sandblasted and acid-etched titanium surfaces after air-particle abrasion with Bioactive glass, Zinc containing bioactive glass or inert glass. An additional aim was to study the attachment and proliferation of human osteoblast-like MC3T3-E1 cells on the same surfaces.

MATERIALS AND METHODS: Sandblasted and acid-etched titanium discs were subjected to air particle abrasion with Bioglass ® 45S5, experimental bioactive glass Zn4 or inert glass. Water contact angles and surface free energy were evaluated. The surfaces were studied with preosteoblastic MC3T3-E1 cells. The cell proliferation and viability were measured biochemically, and their attachment and spreading were visualized by light and electron microscopy.

RESULTS: Air-abrasion with either glasses significantly enhanced the hydrophilicity and surface free energy of the sandblasted and acid-etched titanium discs, $p<0.001$. MC3T3-E1 cell proliferation was significantly higher for substrates air-abraded with 45S5 or Zn4 bioactive glasses, $p<0.001$. Confocal laser scanning microscope images and FE-SEM images showed that MC3T3-E1 cells did not spread on the sandblasted and acid-etched surfaces but attached firmly to the roughened titanium surfaces. For the BAG/inert glass air-abraded surfaces, the cells spread most within 24 h time frame and changed their morphology to more spindle-like when cultured further.

CONCLUSIONS: BAG and inert glass air-abrasion was shown to have a significant effect on the wettability and surface free energy of the surfaces under investigation. Osteoblast cell proliferation was enhanced by Bioglass ® 45S5 and experimental bioactive glass Zn4 air-abrasion compared to inert glass air-abraded discs or sandblasted and acid-etched discs without air-abrasion.

OP 2 EFFECTS OF DIMETHYL SULFOXIDE (DMSO) ON THE DURABILITY OF RESIN-DENTIN BONDS

Anas Aaqel Salim Al-Ani[1], Ikram Aqel Salim[1], M. M. Mutluay[1], L. Tjäderhane[1], A. Tezvergil-Mutluay[1]

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BACKGROUND: One of the main goals in adhesive dentistry is the preservation of hybrid layer, which can be achieved by improving the durability of the resin composite-dentin adhesion as well as the strength of adhesion. Different adhesive strategies have been used to achieve that.

In the last decades, researchers have focused on two different strategies toward improving the durability of adhesion. One strategy focusses on improved penetration and impregnation of the adhesive monomers into the demineralized dentin.

Dimethyl sulfoxide (DMSO; $(CH_3)_2SO$), is a polar aprotic solvent which dissolves polar and nonpolar compounds. It has ability to penetrate biological surfaces. DMSO has recently been suggested to improve the durability and longevity of bonding, by enhancing the penetration of resin monomers in dentin.

OBJECTIVES: The objective of this study was to evaluate the microtensile bond strength (μTBS) and nanoleakage (NL) of bonded resin-dentin interfaces pretreated with various concentrations of dimethyl sulfoxide (DMSO).

MATERIALS AND METHODS: Extracted sound human third molars were used in this study. Occlusal enamel was removed under water cooling and teeth were ground with 600-grit SiC to expose superficial dentin. Dentin surfaces were acid-etched with 37% phosphoric acid and pretreated with various concentrations of DMSO (0.001, 0.01, 0.1, 1, 5, 10, 20%) for 30 s before adhesive application. A single-bottle etch-and-rinse adhesive (SingleBond, 3M ESPE) was applied and restored with resin composite (FILTEK Supreme XTE, 3M ESPE) incrementally to a height of 5mm. After bonding, restored teeth were sectioned to obtain 0.9x0.9 mm beams and were stored in artificial saliva at 37°C for either 24 h or 6 months before μTBS evaluation (n=6 teeth/group). The microtensile bond strength testing (μTBS) was carried out at a crosshead speed of 0.5mm/min. Six beams per tooth were submitted to qualitative nanoleakage evaluation using SEM. The data were analyzed using ANOVA and Tukey's test (α=0.05).

RESULTS: The pretreatment with DMSO had no significant effect on 24 hrs bond strength or nanoleakage. After 6 months storage, μTBS of control group decreased significantly, and also low DMSOconcentrations used (0.001, 0.01%). Whereas 5% and 0.1% DMSO treated groups showed an increase in bond strength. Also DMSO treated groups showed lower nanoleakage compared to control and 0.001% DMSO.

CONCLUSIONS: The use of higher concentrations of DMSO as dentin pretreatment improves the durability of dentin bonding. The use of DMSO as a primer is promising to prolong integrity of resin-dentin interface.

OP 3 PERFORMANCE OF MALDI-TOF MS FOR HUMAN CAPNOCYTOPHAGA IDENTIFICATION

Ahmed Algahawi[1], Eija Könönen[1], Inka Harju[2], Kaisu Rantakokko-Jalava[2], Mervi Gürsoy[1]

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BACKGROUND: The human *Capnocytophaga* species (*C. gingivalis, C. granulosa, C. haemolytica, C. leadbetteri, C. ochracea, C. sputigena*, and *Capnocytophaga* genospecies AHN8471) are Gram-negative, capnophilic, thin rod-shaped bacteria. They are common members of the resident oral microbiota but can also act as opportunistic pathogens in various infections, such as abscesses, septicemia, and adverse pregnancy outcomes.

OBJECTIVE: We aimed to examine, for the first time, the performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for identifying human *Capnocytophaga* species.

MATERIALS AND METHODS: Six type strains and one clinical strain confirmed by 16S rRNA sequencing were used as references. Altogether 106 salivary isolates, originating from 25 healthy women, were presumptively identified as *Capnocytophaga* spp. based on their colony and cell morphologies and routine biochemical tests. All isolates were examined with MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) using the Microflex LT instrument and MALDI Biotyper software version 3.1. Specific cut-off scores were used to determine the genus level (1.700-1.999) and species level (≥2.000) identification. The isolates with a log score of <2.000 were retested using the ethanol-formic acid extraction protocol.

RESULTS: Of the 113 tested isolates, 50 (44.2%) were identified with direct spotting. Spotting was repeated for 36 (31.9%), and ethanol-formic acid extraction was further used for the rest of the isolates. The performance of MALDI-TOF MS to identify *Capnocytophaga* to the genus level was excellent with 100.0% accuracy, while an identification rate of 90.3% was achieved to the species level; 11 strains, representing *C. ochracea* (n=6), *C. haemolytica* (n=2), *C. sputigena* (n=1), *C. leadbetteri* (n=1), and *Capnocytophaga* genospecies AHN8471 (n=1), remained within the low cut-off score. Notably, the latter two species are missing from the current MALDI BioTyper database.

CONCLUSIONS: MALDI-TOF MS constitutes a promising diagnostic tool for identifying human *Capnocytophaga* species. However, an upgrade in the database is needed.

OP 4 INDUCTION OF PLURIPOTENT STEM CELLS FROM ORAL SOURCES FOR THE GENERATION OF MESENCHYMAL STEM CELLS FOR BONE TISSUE REGENERATION

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BACKGROUND: The process of bone tissue engineering involves combining stem cells capable of osteogenic activity with an appropriate scaffold to stimulate the bone regeneration process. These stem cells are in most cases bone marrow mesenchymal stem cells (BM-MSC). However, due to the limited availability of mesenchymal stem cells (MSC) in the bone marrow as well as their reduction in number with age, finding alternative sources is of key importance. Induced pluripotent stem cells (IPSC) have been previously proven to produce MSC. IPSC enable the development of an unlimited source of any type of

human cell needed for therapeutic purposes. These cells can be obtained by reprogramming adult somatic cells into an embryonic like state.

OBJECTIVES: The objectives are as follows: 1) Comparing different sources of human oral (buccal and gingival) fibroblasts to human dermal fibroblasts for efficient reprogramming into IPSC. 2) Comparing the efficiency of IPSC-derived MSC from different sources.

MATERIALS AND METHODS: The fibroblasts were reprogrammed into IPSC, via transfection using episomal plasmids, and images were taken to document and compare their morphology. The expression of pluripotency markers (SOX 2, OCT 4 and NANO G) by the IPSC was measured by RT-PCR. The IPSC were then cultured in differentiation media (89% DMEM, 10% FBS, 1% Pen/Strep) to induce differentiation into MSC like cells (IPS-MSC) after which images were taken to document and compare their morphology.

RESULTS: IPSC were successfully generated from the different fibroblasts, showing typical embryonic stem cell like morphology. A higher number of IPSC colonies developed from the reprogramming of the dermal fibroblasts (11) compared to the gingival (3) and buccal fibroblasts (4), which were comparable. The reprogramming efficiency of obtaining IPSC from the dermal, gingival and buccal fibroblasts was comparable. All the IPSC showed varying levels of expression of SOX 2, OCT 4, and NANO G. Upon differentiation, the IPSC lost the embryonic stem cell like morphology, specific to IPSC, and developed a spindle shaped morphology, typical for MSC (IPS-MSC).

CONCLUSIONS: IPSC can be successfully generated from human dermal, buccal and gingival fibroblasts. According to reprogramming efficiency and IPSC colony development, skin fibroblasts are a superior source for IPSC generation than buccal and gingival fibroblasts, which were comparable to each other. IPSC from human dermal, buccal and gingival fibroblasts can successfully be differentiated into MSC like cells.

OP 5 MODELS FOR PERSONALIZED MEDICINE OF HEAD AND NECK CANCER

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BACKGROUND: Oral cancer patients are mainly treated by surgical removal of the tumor followed by radio-, chemo-, targeted-, and immunotherapy. Efficiency of cancer treatments are not constant and the response varies from patient to patient. This usually leads to several major problems including unnecessary side effects, worse prognosis, and increasing the treatment cost. Because of this, the need for personalized treatment for cancer patients in general and oral cancer specifically, has been growing and became an urgent need. Unfortunately, until now there is no system which could be used to test the efficiency of cancer drugs before giving them to the patient.

OBJECTIVES: We aimed to develop in vitro and in vivo models for testing the efficiency of different cancer treatments using cancer tissue, peripheral blood mononuclear cells (PBMNCs) and serum samples from the same patient.

MATERIALS AND METHODS: We collected tumor tissues during the operation of oral cancers. Samples were washed, minced to 1-2 mm pieces, digested by collagenase I, filtered, and the passed single cells were collected. PBMNCs and serum were isolated from the patients' blood. We set-up two models: the zebrafish assay for testing chemo- and targeted therapy, and the microfluidic chip for testing immunotherapy. In the zebrafish model, isolated tumor cells were injected into the larvae, and the fish were subjected to different

anti-cancer drugs for 3 days. Drugs effectiveness were measured using PCR based technique. In the microfluidic chip, tumor cells were labeled with cell trace far red, mixed with Myogel/fibrin matrix and injected in one channel of the microfluidic device. Isolated PBMNCs were labeled with cell trace violet and injected in the other channel. The device was supplied with the patient serum and different immunotherapeutic agents. Proliferation rate was measured over 3 days by imaging technique.

RESULTS: The two models worked efficiently and showed individual responses to the anti-cancer treatments.

CONCLUSIONS/Significance: This project provides personalized 3D in vitro and in vivo zebrafish models for testing anti-cancer treatments.

OP 6 PROTEOMIC AND PATHOLOGICAL CHARACTERISATION OF SICCA CONTROL SUBJECTS AND PRIMARY SJÖGREN`S SYNDROME PATIENTS REVEALS PROMISING TEAR, SALIVA AND EXTRACELLULAR VESICLE DISEASE BIOMARKERS

Lara A. Aqrawi[1], Hilde Kanli Galtung[2], Eduarda M. Guerreiro[2], Reidun Øvstebø[3], Bernd Thiede[4], Behzod Tashbayev[1,5], Tor Paaske Utheim[2,3,6], Xiangjun Chen[1,5], Øygunn Aass Utheim[3,5], Øyvind Palm[7], Kathrine Skarstein[8,9], Janicke Liaaen Jensen[1]

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BACKGROUND: Mononuclear cell infiltration of exocrine glands, production of Ro/SSA and La/SSB autoantibodies, along with oral and ocular dryness, are characteristic features of primary Sjögren's syndrome (pSS). Non-SS sicca subjects, an underexplored group in relation to pSS, display similar sicca symptoms, with possible mild signs of inflammation in their salivary glands, yet with no serological detection of autoantibody production. We have previously identified novel disease biomarkers in saliva and tears from pSS patients1. In this study, we investigated inflammatory manifestations in the salivary gland tissue, tear fluid and saliva of non-SS subjects, as compared to primary SS (pSS) patients and healthy individuals.

MATERIALS AND METHODS: 15 non-SS, 10 pSS, and 10 healthy subjects were included in the analyses. Histological evaluation of salivary gland biopsies was performed. Liquid chromatography-mass spectrometry (LC-MS) was conducted on tear fluid and stimulated whole saliva, and proteomic biomarker profiles were generated. Extracellular vesicle (EVs) isolation and characterization from both fluids were also combined with LC-MS. The LC-MS data were analysed for quantitative differences between patient and control groups using Scaffold. Database for Annotation, Visualization and Integrated Discovery (DAVID), and Functional Enrichment Analysis Tool (FunRich) were applied for functional analyses.

RESULTS: Histopathological evaluation of salivary gland biopsies showed implications of milder inflammation in non-SS subjects through mononuclear cell infiltration, fibrosis and fatty replacement, as compared to pSS patients. Although unaffected in the non-SS group, upregulation of proinflammatory pathways and proteins involved in ubiquitination (LMO7 and HUWE1) and B cell differentiation (TPD52)

were detected in tear fluid of pSS patients. Moreover, overexpression of proteins STOM, ANXA4, and ANXA1, regulating cellular innate and adaptive immunological pathways, were further identified in EVs from tear fluid of pSS patients. Finally, whole saliva and EVs isolated from whole saliva of pSS patients expressed proteins vital for innate MHC class I cellular regulation (NGAL) and T cell activation (CD44).

CONCLUSIONS: Non-SS sicca subjects may show implications of mild inflammation in their glandular tissue, while their protein profile was strikingly more similar to healthy controls than to pSS patients. Hence, the tear and salivary biomarkers identified could be implemented as potential non-invasive diagnostic tools that may aid in increasing diagnostic accuracy when evaluating non-SS subjects and pSS patients and monitoring disease progression.

OP 7 STRESS DISTRIBUTION OF PARTIALLY VENEERED (SEMI-MONOLITHIC) ZIRCONIA FIXED DENTAL PROSTHESES

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BACKGROUND: Based on previous studies, the structural reliability of veneered zirconia fixed dental prostheses (FDPs) can be enhanced by modifying the framework design. The different design modifications are focused on minimizing the tensile stresses within the weak veneering material by providing supportive structures in the zirconia framework. Previous studies have shown favorable results in the overall strength of monolithic zirconia FDPs. Compared to veneered ones, however, monolithic zirconia FDPs still show lower aesthetic properties. Modified partially veneered (semi-monolithic) designs can provide high aesthetic outcomes using aesthetic veneering materials compared to monolithic restorations while maintaining the strength of monolithic design. The stress distribution in zirconia FDPs with semi-monolithic designs is, however, more complex compared with monolithic designs.

OBJECTIVES: To evaluate influence of framework design on stress distribution within tooth-supported partially veneered FDPs made of zirconia under simulated loads using 3D-FEA.

MATERIALS AND METHODS: For linear FEA, simplified 3D solid models of prepared abutment teeth with different 3-unit FDPs based on designs were created. Five designs—monolithic (control); partially-veneered (semi-monolithic) with 0.3 mm veneer thickness (A); semi-monolithic with 0.5 mm veneer thickness (B); semi-monolithic with 0.5 mm veneer thickness supported with cap design (C), and semi-monolithic with 0.5 mm veneer thickness supported with wave design (D)—were analyzed using FEA. Elastic properties of the components (bone, dentine, cement, translucent zirconia, and veneering porcelain) were gained from standard references for FEA. Simulated static forces (300 N) were applied at oblique direction over occlusal surfaces. Maximum principal stress and shear stress were calculated and analyzed among the different models.

RESULTS: Model C showed lowest maximum principal stress levels in veneering porcelain compared to models A, B, and D. In zirconia framework of model C, however, maximum principal compressive stress levels were higher compared to the other models. Model A had lower maximum principal stress levels in veneering porcelain compared to model B. Maximum principal stress levels were located in veneer

component of models A, B, D whereas were observed at cervical area of zirconia framework of model C. Model A had highest maximum shear stress levels while model D had lowest shear values.

CONCLUSIONS: Framework designs play a significant role in stress distribution of partially veneered zirconia FDPs under loading. The FDP with cap design minimizes maximum principal stress in the 0.5 mm-veneering porcelain. The FDP with a 0.3 mm-veneering porcelain has low maximum principal tensile stress in veneering porcelain but with high maximum shear stress at zirconia-veneer interface. The FDP with wave design minimizes maximum shear stress at the zirconia-veneer interface.

OP 8 NON-SURGICAL ROOT CANAL TREATMENTS AMONG ADULTS IN HELSINKI PUBLIC ORAL HEALTH SERVICE: A 15-YEAR FOLLOW-UP STUDY

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BACKGROUND: Non-surgical root canal treatments (nsRCTs) are assessed to constitute less than 4% of the number of procedures done in Finnish public health sector, but their percentage is more than doubled when calculated by the time spent. Thus, the volume of nsRCTs carried out in public sector would be an interesting topic from the point of view of the use of resources.

OBJECTIVE: To assess the extent of nsRCTs carried out among adult patients in Helsinki public oral health service based on 15-year retrospective observation time.

MATERIAL AND METHODS: Data were extracted from electronic health records of Oral Health Care of the City of Helsinki (OHCH). The inclusion criteria were adult patients of the age of at least 18 years with procedure codes indicating nsRCTs in any of their permanent teeth, except for third molars, from year 2002 to year 2016. Information was available on operator background (general dentist (GD) in public service, GD in private service through purchase agreement with the OHCH, specialized dentist in public service or dental student at the University Dental Clinic), as well as patient-related characteristics (age and gender) and tooth-related factors (tooth type and location in jaw). Data were analyzed with statistical software packages SPSS and Stata for differences between variables.

RESULTS: Based on the inclusion criteria, 83907 adults were identified as having at least one completed nsRCT. The number of root canal-treated teeth was 126999, and the number of non-surgical primary and re-treatments altogether was 131085. Between 2002 and 2016 there was a slight increase in the average yearly number of nsRCTs, ranging from 6159 to 10370. Before 2006 majority of the nsRCTs were performed by public general dentists, but since 2007 they have been performed for the most part by private general dentists with service purchases. The most common root canal-treated teeth were mandibular molars (27.8%), followed-by maxillary molars (21.6%) and maxillary premolars (21.0%).

CONCLUSIONS: The yearly magnitude of the procedures shows a slightly increasing trend in the number of nsRCTs, which indicates a more substantial increase in the need of public oral health care in terms of time and the use of resources. The purchases of nsRCTs from private sector through purchase agreements

seem to answer to the increasing demand.

OP 9 NON-SURGICAL PERIODONTAL TREATMENT HAS IMPACT ON SALIVARY CHEMOKINE LEVELS

Maria Anastasia Grande[1], Daniel Belstrøm[1], Christian Damgaard[1], Eija Könönen[2], Mervi Gürsoy[2], Palle Holmstrup[1], Ulvi K. Gürsoy[2]

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BACKGROUND: During wound healing, macrophages regulate inflammation, remove apoptotic cells, and promote tissue maturation. The aim of the present study was to determine how non-surgical periodontal treatment influences salivary monocyte/macrophage-related chemokine levels. We tested the hypothesis that non-surgical periodontal treatment has impact on salivary levels of Monocyte Chemoattractant Protein-1-4 (MCP-1, MCP-2, MCP-3 and MCP-4), Macrophage-Derived Chemokine (MDC), Macrophage Migration Inhibitory Factor (MIF), Monokine induced by IFN-gamma (MIG), Macrophage Inflammatory Protein-1α (MIP-1α), and Interferon Inducible Protein-10 (IP-10), and that changes in salivary levels of the chemokines correlate with clinical parameters.

MATERIAL AND METHODS: Twenty-five patients with moderate to severe chronic periodontitis, as defined by American Academy of Periodontology (1999 classification), completed the study. Stimulated saliva samples were collected at baseline and 2, 6 and 12 weeks after non-surgical periodontal treatment. Salivary levels of MCP-1 (CCL2), MCP-2 (CCL8), MCP-3 (CCL7), MCP-4 (CCL13), MDC (CCL22), MIF, MIG (CXCL9), MIP-1α (CCL3), and IP-10 (CXCL10) were quantified using the Luminex® xMAP™ technique, according to manufacturers' protocol. The chemokine levels, before and after periodontal treatment, were compared using Friedman's test, and correlation of chemokines was tested using Spearman's signed rank test. Trial registration number: NCT02913248 at clinicaltrials.gov.

RESULTS: Periodontal treatment improved all clinical parameters and was followed by significant alterations in mean salivary levels of MCP-2, MCP-3, MDC, MIG and MIP-1α. A significant increase in levels of MCP-2, MCP-3, MCP-4, MDC, and MIG was observed in week 12 compared to week 2 and 6 respectively. Significant correlations of salivary levels of MCP-1, MCP-2, MCP-3, MCP-4, IP-10 with clinical parameters such as bleeding on probing (BOP) and probing depth (PD) were observed.

CONCLUSION: Elevated salivary chemoattractant levels may indicate that monocytes and macrophages take part in late tissue maturation after periodontal wound healing. Further studies are needed to reveal if alterations in salivary chemokine levels precede clinical signs of periodontitis.

OP 10 CLUSTERED TiO_2 NANOTUBULAR SURFACE WITH PDGF-BB COVALENT MODIFICATION ENHANCES BONE REGENERATION WITH MARKEDLY BOOSTED OSTEOCALCIN EXPRESSION

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BACKGROUND: A multitude of surface structures have been developed to improve the osseo-integration of titanium (Ti)-based implants. Unfortunately, most of these surface topographies failed to represent the spatial hierarchical structure of natural bone tissue due to limitations in either the sole micron- or nano-scale, respectively.

OBJECTIVES: To establish an osteo-inductive surface structure with platelet derived growth factor (PDGF)-BB covalent modification on Ti implant. This compound surface structure not only mimic the hierarchical micro-/nano-structure of natural bone tissue but also develop a bioactive "niche" to facilitate the osteogenesis-related functions both *in vitro* and *in vivo*.

MATERIALS AND METHODS: Clustered TiO_2 nanotubular surface were textured via a modified anodization with a perchloric acid-based electrolyte and programmed voltage alteration (30 V-20 V-10 V in sequence) and denoted as NT. NT surface was coated with 11-hydroxyundecylphosphonic acid (PhoA) monolayer before carbonyldiimidazole (CDI) activation to reduce the negative effects of unwanted steric hindrance. Then, low-temperature hydrothermal method was employed to link PDGF-BB on activated CDI structure. Such PDGF-BB modified NT sample was labelled as NTPCP. Comprehensive *in vitro* and *in vivo* experiments were conducted to examine the surface characteristics, cytotoxicity, and osteo-inductive activity (using human bone marrow mesenchymal stem cells (bMSCs) and rat implantation model) of the modified Ti surfaces with PDGF-BB modification.

RESULTS: Anodization lead to the rapid growth of TiO_2 nanotubes and made them twisted to form a clustered TiO_2 nanotubular structure observed on NT samples. Such surface structure highly mimicked the spatial hierarchical micro/nano structure of bone collagen. X-ray photoelectron spectroscopy (XPS) analysis proved that PDGF-BB were covalently modified on NT surface (NTPCP). NTPCP provided a 3D biomimetic structure to host cells, with negligible cytotoxicity and satisfactory bio-activity for significantly enhancing osteogenesis-related functions of human bMSCs (including attachment, early-stage proliferation, extracellular matrix synthesis and mineralization). Remarkably, we observed significantly elevated expression of osteocalcin (OCN), which mirrored prominent bone formation around the NTPCP implants in a rat model.

CONCLUSIONS: In this study, we constructed a novel strategy of compound surface modification to improve osseointegration of Ti implants. Considering the regulatory effects of OCN on glucose homeostasis, NTPCP can be promisingly applied in diabetic patients in the future.

OP 11 PROTEOMICS OF SALIVA, PLASMA AND SALIVARY GLAND TISSUE IN PATIENTS WITH PRIMARY SJÖGREN`S SYNDROME

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BACKGROUND: Primary Sjögren's syndrome (pSS) is characterized by an immune-mediated destruction and chronic dysfunction of exocrine glands, including salivary glands. As the glands are directly involved in the pathology of the disease, it is likely that saliva in addition to blood and salivary gland tissue itself reflect the underlying pathogenic mechanisms of glandular dysfunction. Previous studies indicate that patients with pSS in comparison to healthy subjects display a distinct salivary proteome that reflects the glandular pathobiology.

OBJECTIVES: To characterize and compare the proteome in whole saliva, plasma and salivary gland tissue from patients with pSS and patients having symptoms of pSS, but not fulfilling the classification criteria (non-pSS).

MATERIALS AND METHODS: Forty patients were enrolled in the study of whom 19 fulfilled the American-European Consensus Classification Criteria for pSS and 21 did not (non-pSS). Chewing-stimulated whole saliva, labial salivary gland tissue and plasma samples were collected for proteomic analysis. Samples were lysed with buffer and digested with LysC, followed by LC-MS/MS analysis. Rawfiles were processed with MaxQuant. The integrated search engine and a reversed database approach applying a 1% FDR at both peptide and protein level was used. The data was searched against the Uniprot human reference proteome database. Further statistical analysis was done in Perseus software.

RESULTS: A total number of 1,013, 219 and 3,166 proteins were identified in saliva, plasma and tissue, respectively. In saliva, 44 proteins were significantly different expressed in patients with pSS than in non-pSS. Among those, β-2-microglobulin was up-regulated, whereas histatins, cystatins and mucin-7 were down-regulated. Proteins driving the separation in principal component analysis were primarily related to salivary secretion in non-pSS patients, whereas proteins belonging to complement and coagulation cascades were associated with pSS (KEGG pathway database). There were no significant difference between the patient groups in terms of protein expression in plasma and salivary gland tissue.

CONCLUSIONS: Our findings indicate that patients with pSS display a distinct salivary proteome, which enables differentiation from non-pSS patients. Protein expression in plasma and salivary gland tissue in patients with pSS resembles that of non-pSS.

OP 12 PREVALENCE AND CHARACTERISTICS OF OSTEOARTHRITIS IN THE TEMPOROMANDIBULAR JOINT IN NORWEGIAN 65 YEAR OLDS. A CBCT STUDY.

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BACKGROUND: Osteoarthritis (OA) is the most common joint disease, hence frequently found in the temporomandibular joint (TMJ). It is a complex, gender- and age-related disease with inflammatory mediators released by cartilage, bone, and synovium. Severity of the OA related osseous changes increases by age, also in the TMJ. Even so, the prevalence of osteoarthritis in the temporomandibular joints in the aging urban population is largely unknown. Due to an increasing number of elderly, dental health care services will face new challenges in the decades to come. In order to predict TMJ related challenges in the aging population in the future, it is beneficial to document the prevalence of the TMJ-OA in this population. The intention of a follow up study, will further contribute to valuable knowledge of both incidence and progression of TMJ-OA.

OBJECTIVES: To determine the prevalence of and characterize clinical and radiological findings of osteoarthritis (OA) in the temporomandibular joints (TMJs) by means of cone beam computed tomography (CBCT) and validated diagnostic criteria in 65-year old Oslo citizens.

MATERIAL AND METHODS: Participants will randomly be recruited from the larger epidemiological study “Oral health of 65-year-old Oslo citizens”, which will consist of a representative sample of 450 65-year-olds in Oslo. The participants will be recruited independently of TMJ related symptoms.

The TMJs of the participants will be examined with CBCT (Accuitomo, Morita CBCT). Images will be analyzed for bone change characteristics and each joint will be diagnosed with either OA, no OA or as indeterminate for OA. The image analysis criteria developed for the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) will be used. Frequencies of bone changes, joint diagnoses and severity grades will be calculated, as well as kappa values for observer agreement.

Two simple tools from the Diagnostic Criteria for TMD (DC/TMD) Axis I protocol will be used for assessment of TMJ-related symptoms: TMD pain screener, and Diagnostic criteria for clinical TMJ-OA.

Data collection started in April 2019 and is expected to be completed by the end of the year. Estimated study sample is 100 participants.

RESULTS: Preliminary interpretation of the CBCT images shows that mild TMJ-OA is common in 65-year olds in Oslo. Despite these results, the self-reporting indicate low burden of TMJ related symptoms in this population. Preliminary results will be presented.

OP 13 C-DI-AMP SIGNALLING IN STREPTOCOCCUS MITIS

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BACKGROUND: *Streptococcus mitis* (*S. mitis*) is an oral commensal that normally lives in harmony with the human host and mainly causes disease in immunocompromised individuals. Cyclic di adenosine monophosphate (c-di-AMP) is a second messenger, relaying environmental signals into cellular responses. C-di-AMP is produced by diadenylate cyclases and broken down by phosphodiesterases. It controls a multitude of traits in different bacteria, for example potassium homeostasis, biofilm formation and sensitivity to antibiotics. This signalling system has never been studied in *S. mitis*, but we hypothesise that it is involved in regulation of the commensal lifestyle of these bacteria.

OBJECTIVES: The main objective is to characterize the c-di-AMP signalling system in *S. mitis*. This will be achieved by understanding the role of c-di-AMP in the control of antibiotic susceptibility, survival under stress, growth of bacteria, metabolism and adherence to human cells.

MATERIALS AND METHODS: Knock out mutants of each of the three genes hypothesised to be involved in c-di-AMP signalling (*dacA, pde1 and pde2)* were made in *S. mitis* CCUG 31611 by markerless gene deletion. Assays were performed to identify any c-di-AMP regulated phenotypes. These included growth in rich medium and in defined media, deficient in key components such as potassium and amino acids. The wild type (WT) and knock out mutants were tested against a panel of antibiotics to determine effects of c-di-AMP regulation on antibiotic susceptibility. In addition, the mutants and WT were screened against different types of stress, including heat, low pH, and UV-light. Carbohydrate metabolism was investigated using ^{14}C-labeled glucose in the presence or absence of potassium, using HPLC.

RESULTS: Bioinformatics analysis of the *S. mitis* genome identified one and two genes predicted to code for a diadenylate cyclase and phosphodiesterases, respectively. The successful knock out of these genes were confirmed by sequencing. The most pronounced effect of the knock out was observed in the *Pde2*-deletion mutant that displayed slower growth and low glucose metabolism. Wild type phenotypes were restored following knock-back of the genes into the respective mutants.

CONCLUSIONS: Knock out of the gene coding for the putative phosphodiesterase Pde2 gave effects on both colony morphology, growth and metabolism. These effects were reversible by knocking back the genes. Knocking out the gene could potentially lead to either an accumulation of c-di-AMP or the intermediary metabolite pApA. Further studies are needed to elucidate the mechanisms behind the observed effects. This will be done by isolating the proteins and characterizing their enzymatic activities.

OP 14 MODULATION OF MESENCHYMAL STROMAL CELLS INFLAMMATORY RESPONSES BY BONE SUBSTITUTES

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BACKGROUND: Mesenchymal stromal cells (MSCs) are increasingly being used in combination with bone substitutes (BS) for craniofacial bone regeneration[1]. Although their mechanism of action is not well defined, emerging evidence suggests a more immunomodulatory function of MSCs rather than direct osteogenic differentiation[2]. Moreover, little is known about the effects of inflammation on MSCs and BS.

OBJECTIVES: To investigate the effects of inflammatory stimuli on (1) early cellular responses, and (2) subsequent osteogenic differentiation of MSCs seeded on BS.

MATERIALS AND METHODS: In two *in vitro* experimental setups, a biphasic CaP-based BS was seeded with human MSCs under pro-inflammatory stimulation (IL–1beta, TNF–alpha; BC^+ group) for up to 72 h, followed by osteogenic induction for 14 d. Control groups included non-stimulated (BC^- groups) and/or plastic-cultured (2-D) MSCs. Expressions of genes related to wound healing, inflammation/immunomodulation, osteogenesis and osteoclastogenesis were assessed at different time points via RT-PCR. Expressions of selected proteins were measured via ELISA and specific biochemical assays. Experiments were performed with at least three replicates and statistically analyzed.

RESULTS: Overall, results indicated that the combination of BS and inflammatory stimulation (BC^+ groups) significantly upregulated several genes and proteins related to wound healing (TGF-beta, BMP–2, VEGF), immunomodulation (IL–6, –8, –10) and osteoclastogenesis (RANKL/OPG) *vs.* BC^- groups over 72 h. However, both BC^+ and BC^- groups showed significantly downregulated genes (RUNX2, ALP, COL, BSP, OCN) and alkaline phosphatase activity related to osteogenic differentiation of MSCs *vs.* 2-D controls over 14 d.

CONCLUSIONS: The combination of BS and a pro-inflammatory microenvironment induces an immunomodulatory rather than an osteogenic phenotype of MSCs. Further studies of interactions between MSCs, immune cells and BS are currently being performed to validate these findings.

OP 15 NON-SJÖGREN`S SYNDROME SICCA PATIENTS PRESENT SEVERELY REDUCED GENERAL AND ORAL HEALTH-RELATED QUALITY OF LIFE

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OBJECTIVE: To compare general health- and oral health- related quality of life in primary Sjögren´s syndrome (pSS) patients with that of age- and gender matched non-Sjögren´s sicca syndrome (non-SS) and

healthy control subjects.

MATERIALS AND METHODS: Sixty patients with pSS (age: 54±13 years), 22 non-SS patients with sicca symptoms and findings, but negative for anti-SSA autoantibodies and salivary gland foci (52±10 years), and 43 healthy control subjects (49±14 years) were included in this cross-sectional study. Subjects underwent extensive dry eye work-up including Ocular Surface Disease Index (OSDI) questionnaire, tear osmolarity (TO), tear film break-up time (TFBUT), Schirmer test (ST), and ocular surface staining (OSS, Oxford grading scheme). Oral examinations included subjective oral dryness score (SXI), unstimulated whole saliva (UWS), chewing-stimulated whole saliva (SWS), and evaluation of clinical oral dryness score (CODS). The 36-Item Short Form Survey (SF-36) was used to assess general health related quality of life (GHRQoL) and expressed as physical component score (PCS) and mental component score (MCS). The Oral Health Impact Profile (OHIP-14) was used to evaluate oral health related quality of life (OHRQoL). Kruskal-Wallis H test was used for intergroup comparison. P values indicate difference between the groups.

RESULTS: The SXI was highest in non-SS patients (12.3±1.9) as compared to pSS (11.8±2.5, $p<0.001$) and healthy controls (6.2±0.4, $p<0.001$). The non-SS group displayed worse GHRQoL compared to pSS group (PCS: 31.5±10.2 vs 44.1±10.2 ($p<0.001$) and MCS: 41.4±11.6 and 46.2±10.3, $p<0.001$). Similarly, the non-SS group had worse OHRQoL (OHIP-14: 13.5±10.5 vs 18.6±13.9, $p<0.001$) than pSS group. However, compared to pSS group, the non-SS subjects had less pronounced objective findings of ocular dryness (TO: 328.2±23.2 vs 326.7±22.3, $p=0.04$, TFBUT: 2.3±2.1 vs 3.1±2.1, $p<0.001$, ST: 5.7±5.6 vs 12.9±9.6 $p<0,001$and OSS: 3.2±2.6 vs 1.3±1.2, $p<0.001$), and oral dryness (CODS: 5.0±1.9 vs 4.2±2.1, $p<0.001$, UWS: 1.3±1.2 vs 1.7±1.2, $p<0.001$, SWS: 3.5±2.8 vs 4.8±1.7, $p<0.001$). Healthy controls showed normal parameters.

CONCLUSIONS: Despite having clinically milder ocular signs of dry eye disease and less pronounced findings of oral dryness, non-SS patients may have more subjective complaints of dry eye and dry mouth, and suffer from reduced general and oral health related quality of life, as compared to patients fulfilling the classification criteria for pSS. Consequently, clinicians should pay appropriate attention to sicca patients no matter they fulfil the classification criteria or not.

OP 16 HUMAN TUMOR-DERIVED MATRIX IMPROVES PREDICTABILITY OF HEAD AND NECK CANCER DRUG TESTING

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BACKGROUND: Head and neck squamous cell carcinoma (HNSCC) represents the sixth most common cancer worldwide, with a relatively low survival rate of around 50%. Current pre-clinical anti-cancer drug testing carries a low predictive value since only 5% of compounds showing efficacy in *in vitro* tests are licensed following clinical trials. Therefore, improved predictive *in vitro* methods are urgently needed.

OBJECTIVES: Cancer research is moving towards 3D cell culture *in vitro* models. We have developed the human tumor leiomyoma-derived matrix “Myogel” to better mimic the human tumor microenvironment

(TME). We propose that Myogel would provide an appropriate TME for testing the response of human HNSCC cells to various drugs.

MATERIALS AND METHODS: We selected 12 HNSCC cell lines and 19 anti-cancer compounds targeting the EGFR, PI3K-mTOR and MAPK signaling pathways. We applied a high-throughput drug screening assay under five different conditions: cells in plastic wells, on top and embedded in mouse tumor-derived Matrigel® or Myogel. We compared the efficacy of the anti-cancer drugs tested *in vitro* with the response rate of the same drugs in the clinical trials of the HNSCC patients.

RESULTS: The cell viability readout revealed clearly differential EGFR and MEK inhibitor effects between Myogel and the other test conditions. However, cell lines responded similarly to PI3K/mTOR inhibitors independent of the test conditions. Importantly, Myogel in the drug screening of HNSCC cells yielded the highest similarity to the response rates of those drugs which had been tested in clinical trials.

CONCLUSIONS: Our data suggest that human TME mimicking Myogel clearly improves the predictability of anti-cancer drug testing of HNSCC cell lines. This technique would most probably reduce the number of false-positive pre-clinical results, the cost of drug development, and the unnecessary suffering of cancer patients.

2. Poster presentations

____ Presenting author

P 1 ADIPOSE-DERIVED VERSUS BONE MARROW MESENCHYMAL STEM CELLS FOR BONE TISSUE ENGINEERING

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BACKGROUND: Adipose-derived stem cells (ASC) have been proposed as an alternative to bone marrow mesenchymal stem cells (BMSC) in many applications. Studies comparing human ASC and BMSC for bone tissue engineering have shown conflicting results regarding superiority of stem cells from one source over the other. In these studies, ASC were compared to BMSC obtained from different donors. Variations between donors might have influenced the properties of the cells and consequently contribute to these inconsistent results.

OBJECTIVES: This work aimed to compare the bone forming capacity of donor-matched human ASC and BMSC.

MATERIALS AND METHODS: Donor-matched human ASC and BMSC were isolated, expanded and seeded onto poly(L-lactide-co-ε-caprolactone) scaffolds. Osteogenic differentiation of the cells was investigated *in vitro*. Further, cell/scaffold constructs and scaffolds without cells (control) were implanted subcutaneously in immunodeficient mice and in calvarial bone defects in immunodeficient rats. Bone formation was evaluated at the gene level, radiographically and histologically.

RESULTS: ASC and BMSC attached to the scaffolds and demonstrated osteogenic differentiation *in vitro* with early increased alkaline phosphatase activity and mineralization after 21 days. However, higher alkaline phosphatase activity was detected in BMSC. The subcutaneously implanted ASC and BMSC constructs showed upregulated osteogenesis-related genes after 2 weeks, with formation of extensive collagen matrix after 10 weeks. However, no apparent mineralization was detected. In calvarial defects with ASC or BMSC, the osteogenesis-related genes were upregulated after 4 weeks. Higher cellular activity was detected in defects treated with BMSC than ASC after 4 and 12 weeks. Defects with BMSC had greater bone formation than defects with ASC after 4 weeks, but after 12 and 24 weeks, comparable bone formation was observed.

CONCLUSIONS: Although ASC demonstrated the capacity to form bone, rate of bone formation with ASC was slower than BMSC. Accordingly, BMSC may be more preferable for bone tissue engineering applications.

P 2 INTERLEUKIN 17F EXHIBITS ANTITUMOUR EFFECTS IN ORAL TONGUE CANCER

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BACKGROUND: We recently showed that extracellular interleukin-17F (IL-17F) correlates with better disease-specific survival in oral tongue squamous cell carcinoma (OTSCC) patients. However, the underlying mechanisms of such effect remain obscure.

MATERIALS AND METHODS: we used qRT-PCR to assess the expression of IL-17F and its receptors (IL-17RA and IL-17RC) in two OTSCC cell lines (HSC-3 and SCC-25) and in normal human oral keratinocytes (HOKs). IL-17F effects on cancer cell proliferation, migration, and invasion were studied using a live-imaging IncuCyte system, and a Caspase-3/7 reagent was used for testing apoptosis. 3D tumor spheroids were utilized to assess the impact of IL-17F on invasion with or without cancer-associated fibroblasts (CAFs). Tube-formation assays were used to examine the effects of IL-17F on angiogenesis using human umbilical vein endothelial cells (HUVEC).

RESULTS: OTSCC cells express low levels of IL-17F, IL-17RA, and IL-17RC mRNA compared with HOKs. IL-17F inhibited cell proliferation and random migration of highly invasive HSC-3 cells. CAFs promoted OTSCC invasion in tumor spheroids, whereas IL-17F eliminated such effect. IL-17F suppressed HUVEC tube formation in a dose-dependent manner.

CONCLUSIONS: Collectively, we suggest that IL-17F counteracts the pro-tumorigenic activity in OTSCC. Due to its downregulation in tumor cells and inhibitory activity in in vitro cancer models, targeting IL-17F or its regulatory pathways could lead to promising immunotherapeutic strategies against OTSCC.

P 3 TUMOR BUDDING: AN EARLY SIGN OF INVASION, METASTASIS AND POOR PROGNOSIS IN ORAL CANCER

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BACKGROUND: Tumor budding, defined as the presence of single cancer cell(s) or cluster(s) of less than five cancer cells at the invasive front, has been reported in different epithelial cancers as a promising prognostic feature. Tumor budding reveals dissociative behavior of cancer cells at the invasive front.

OBJECTIVES: We sought to evaluate the clinical significance of tumor budding in multi-institutional study of early-stage oral tongue cancer. We also systematically reviewed the literature for all published studies in oral squamous cell carcinoma as well as in other cancers of head and neck.

MATERIALS AND METHODS: We conducted a multicenter retrospective study of 311 cases treated for oral tongue squamous cell carcinoma to evaluate the prognostic significance of tumor budding. In addition, we conducted a systematic review on tumor budding in head and neck cancer.

RESULTS: Tumor budding was a prognosticator for cancer-related mortality in oral tongue cancer with a hazard ratio of 2.04 and 95% confidence interval (CI), 1.17-3.55; $P = 0.01$. An association with

lymph node metastasis (odds ratio 7.08, 95% CI 1.75-28.73) was reported for oral squamous cell carcinoma. The published studies have shown that tumor budding is an important prognosticator in other subsites of head and neck cancer.

CONCLUSIONS: Tumor budding has a prominent prognostic power in early oral tongue cancer. Similar finding has been reported in other tumors of head and neck. Therefore, tumor budding would have a significant role in selection of the treatment strategy.

P 4 MOTOR CONTROL STRATEGIES IN RELATION TO UNPREDICTABLE LOAD CHANGES DURING ISOMETRIC FORCE CONTROL TASKS

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BACKGROUND: A skilled object manipulation task depends to a large extent on the ability of the central nervous system (CNS) to assimilate and interpret sensory information relevant to the task. Skilled object manipulation also relies on the ability of the CNS to predict information about the objects physical properties and to augment this information for developing suitable motor programs required for successful task completion. Errors in the prediction of this information leads to discrepancies in developing/adjusting appropriate motor programs and as a result failure of the task objectives.

OBJECTIVES: The study investigates the motor control strategies of the oral motor system (OMS) during a sequential and non-sequential load changes and compare the employed strategies with the hand motor system (HMS).

MATERIALS AND METHODS: Seventeen healthy adults (age: 24.0 ± 4.3 years) performed a standardized isometric force control task. The task involved holding and pulling a force transducer either with the anterior teeth or the fingers while a series of loads were added to a hook attached to the force transducer. The loads to the hook were attached in a sequential and non-sequential manner. The force profile obtained during the task was divided into two time-segments; an "initial segment" representing the first two seconds of the force profile and a "latter segment" representing the remaining force profile. The peak force during the initial segment, and the holding force along with the variability (coefficient of variation) of the holding force during the latter segment were subjected to repeated measure ANOVAs and analyzed.

RESULTS: The results showed that the OMS applied higher peak force during the initial segment than the HMS ($P<0.001$). Further, during the latter segment the OMS applied higher holding force ($P<0.001$) and higher force variability ($P=0.002$) than the HMS. However, for both the OMS and the HMS, there were no significant differences in either peak force ($P=0.27$), holding force ($P=0.166$) or force variability ($P=0.439$) between the sequential and non-sequential load changes.

CONCLUSIONS: The differences in force magnitude and force variability between the OMS and the HMS are attributed to the structural differences between the two motor systems. Further, the results

indicate that sequential and non-sequential load changes did not impair the force control during the isometric motor control task of the OMS and the HMS.

P 5 ANTIMICROBIAL POTENTIAL OF STRONTIUM ON DIFFERENT BACTERIA ASSOCIATED WITH PERI-IMPLANT DISEASE

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BACKGROUND: Strontium (Sr) has been shown to exert some antimicrobial action against various oral pathogens. However, the possible antibacterial properties of Sr against bacteria associated with implant biomaterial infections have not been evaluated.

OBJECTIVE: To investigate the antibacterial potential of Sr against different oral bacteria associated with implant biomaterial associated infections.

MATERIALS AND METHODS: Six different concentrations of SrOH (100, 10, 1, 0.1, 0.01 and 0.001mM) were prepared and tested against five oral bacteria commonly associated with biomaterial infections: *Streptococcus mitis (S. mitis), Staphylococcus epidermidis (S. epidermidis), Porphyromonas gingivalis (P. gingivalis), Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans), Fusobacterium nucleatum (F. nucleatum), Entrobacteria.* Agar diffusion test, minimal inhibitory concentration (MIC) and biofilm viability assays were applied, and each concentration was tested.

RESULTS: 0.01, 0.1 and 1 mM showed zones of inhibition against *P. gingivalis* with a mean diameter of (15, 24, 21 mm respectively). 10 mM was the MIC for all species tested ($p < 0.01$). In the biofilm viability assay total viable biofilm cell count was (25, 42, 0, 40 and 22 %) at 10 mM Sr compared to (75, 96, 86, 92 and 49%) in the control for *S. mitis*, *S. epidermidis*, *A. actinomycetemcomitans*, *Enterobacteria* and *P. gingivals*, respectively.

CONCLUSION: Sr has antimicrobial properties directed towards bacteria associated with implant biomaterial associated infections.

P 6 CROSS-LINKED GELATIN-NANOCELLULOSE SCAFFOLDS FOR BONE TISSUE ENGINEERING

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BACKGROUND AND OBJECTIVES: Scaffolds for bone tissue engineering must be biocompatible to ensure acceptance from the host tissues, to support regeneration of new bone. Gelatin is biocompatible, and interestingly, it has been found that addition of wood-based cellulose nanofiber (CNF) to gelatin matrix, improves the mechanical properties and degradation rate of gelatin scaffolds. The aim of this study was to investigate the cellular and tissue responses to gelatin-CNF scaffolds, cross-linked chemically with genipin and hexamethylenediamine (HMDA), and followed by dehydrothermal treatment (DHT).

MATERIALS AND METHODS: Nanocellulose hydrogels were produced by TEMPO-mediated oxidation. CNF were then blended with gelatin and cross-linked by genipin, HMDA and DHT. To produce 3D porous scaffolds, the prepared CNF and gelatin-CNF were freeze-dried for 24h. Interaction with human bone marrow mesenchymal stem cells in terms of cytotoxicity, proliferation, inflammation and osteogenic differentiation was investigated. Furthermore, scaffolds were implanted subcutaneously in rats for 90 days followed by histological analysis.

RESULTS: The *in vitro* results demonstrated that both scaffolds were nontoxic and maintained cell viability and proliferation up to 7 days. Osteogenic-related gene expressions were comparable in both groups after 7 and 21 days. The mRNA expression of inflammatory cytokines by the cells grown on gelatin-CNF scaffolds was lower after 7 days of culture. The gelatin-CNF scaffolds had a higher degree of degradation *in vivo* after 90 days. At day 4, histologically both scaffolds showed inflammatory cell infiltration into the pores; however, more cells invaded the gelatin-CNF scaffolds. At 90 days, both scaffolds exhibited thin fibrous capsule, and were filled with newly formed collagen matrix, fibroblasts and blood vessels.

CONCLUSIONS: The inclusion of CNF into gelatin matrix followed by cross-linking treatment with genipin, HMDA and DHT is safe and suitable strategy to fabricate biocompatible and biodegradable scaffolds for bone tissue engineering.

P 7 EVALUATION OF ORAL AND OCULAR PRESENTATIONS OF PRIMARY SJÖGREN SYNDROME

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BACKGROUND: Primary Sjögren syndrome (SS) is commonly associated with dry mouth and dry eye, and labial salivary gland biopsy (LSGB) is an important contributor to diagnosis of SS. The correlation between clinical presentation of xerostomia and xerophthalmia with positive focus score and existence of germinal center in LSGB remains to be explored.

OBJECTIVES: To compare clinical oral and ocular presentations of pSS with non-SS sicca patients, as well as to relate clinical parameters with histopathological findings in LSGB.

MATERIALS AND METHODS: Twenty-one pSS patients (20 females and 1 male, mean age: 49±14.8 years) and 22 female non-Sjögren´s syndrome (non-SS) subjects with sicca symptoms (age: 52.1±10.4 years) were included in the current study. Based on histopathological findings in LSGB, the pSS patients were further divided into positive and negative focus score (FS) (FS+, FS≥1, n=16; and FS-, FS<1, n=5) groups, as well as positive and negative germinal center (GC+, n=10; and GC-, n=11) groups. All non-SS subjects had negative histopathological findings. Clinically, oral (Summated Xerostomia Inventory, SXI) and ocular (McMonnies Dry Eye questionnaire, MDEIS, and Ocular Surface Disease Index, OSDI) subjective complaints were recorded. Objective findings including clinical oral dryness scores (CODS), unstimulated and stimulated saliva secretion rates (UWS/SWS),

Schirmer I test, tear osmolarity, tear film break-up time (TFBUT), and ocular surface staining (OSS) were determined. Kruskal-Wallis U test was used in the intergroup comparison, while the Mann-Whitney U test was applied to determine differences between two groups. A p-value of <0.05 was considered significant.

RESULTS: non-SS subjects had higher OSDI than FS- and FS+ patients (51.7±24.0, 27.1±21.9, and 24.2±18.6, respectively), despite higher Schirmer I test than FS+ patients (9.5±6.7 vs. 4.6±2.9 mm/5min, p=0.006), and longer TFBUT than FS- patients (2.8±1.9 vs. 1.0±0.0 s, p=0.028). FS+ patients also had higher candida score than non-SS subjects (1.8±1.1 vs. 0.8±1.0, p=0.024). No significant differences were detected between FS+ and FS- patients, except that FS+ patients had longer TFBUT than FS- patients (2.1±0.9 vs. 1.0±0.0 s, p=0.019). Based on GC, non-SS subjects had higher OSDI score than GC- and GC+ patients (51.7±24.0, 20.7±16.8, and 29.5±20.8, respectively), and higher Schirmer I test (9.5±6.7 vs. 3.9±1.9 mm/5min, p=0.006) and less OSS (1.8±1.5 vs. 4.6±2.7, p=0.021) than GC+ patients. No significant difference was detected between GC+ and GC- pSS patients.

CONCLUSIONS: pSS patients, specially FS+ and GC+ patients showed reduced tear production, more ocular surface damage, higher candida score compared to non-SS subjects. However, current study did not show positive correlation between severity of clinical dry mouth and dry eye presentation in pSS patients and their histopathological findings in LSGB. Due to limited sample size, future study with larger sample size is warranted.

P 8 ORAL SQUAMOUS CELL CARCINOMAS EXPRESSING HIGHER LEVELS OF S100A14 CORRELATE WITH PROGNOSIS

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BACKGROUND: Altered expression of S100A14 has been reported in human cancers, but its biological role in tumorigenesis is not fully understood. This study aimed to investigate the expression profile and clinical significance of S100A14 in oral squamous cell carcinoma (OSCC).

MATERIALS AND METHODS: S100A14 protein expression was examined in formalin-fixed paraffin embedded (FFPE) specimens of OSCC (n=175), Norway (n=106) and Nepal (n=69) by using immunohistochemistry (IHC). S100A14 was over-expressed and knocked-down in OSCC-derived (CaLH3 and VB6) cells by employing retroviral constructs to investigate its effects on the expression of differentiation markers.

RESULTS: Overall, S100A14 protein was found to be down-regulated at the invading front/island as compared to the tumor center. Low S100A14 protein levels at the invading front/island in OSCC significantly correlated with reduced 10-year overall survival and poor tumor differentiation. Analysis of two external OSCC microarray datasets showed a positive correlation between the mRNA expression levels of *S100A14* and keratinocyte differentiation markers. Corroborating these findings, retroviral mediated S100A14 over-expression and knock-down in CaLH3 and VB6 cells led to respective up- and down-regulation of differentiation markers.

CONCLUSIONS: These results indicate that S100A14 is a differentiation promoting protein and might function as a tumor suppressor in OSCC.

P 9 ORAL HEALTH OF 65-YEAR-OLDS IN OSLO

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We intend to carry out an epidemiological study to assess the oral health status in a representative sample of 450 65-year-olds in Oslo. The overall aim of the study is to investigate oral health conditions (dental caries, periodontal, endodontic conditions, dry mouth, taste and smell) in the aging urban population and to explore important oral disease determinants using a multivariate approach including relevant biological and socio-behavioural risk indicators.

BACKGROUND: Due to an increasing number of elderly maintaining their own teeth, dental health care services will face new challenges in the decades to come. In order to predict the future oral health needs in the aging population, it is necessary to have information about the extent of oral diseases among the elderly. However, data on oral diseases and conditions among Norwegian middle-aged adults, especially from the Southern part of Norway, are scarce. The present interdisciplinary epidemiological study therefore intends to investigate oral health parameters in a representative sample of 65-year olds in Oslo. The plan is to follow up participants after 5 and 10 years in order to document expected longitudinal changes.

OBJECTIVES: To investigate oral health conditions (dental caries, periodontal, endodontic conditions and dental occlusion) among 65-year-old Oslo citizens; to investigate prevalence of dry mouth and associated factors in this group; to investigate the ability to taste and smell in this group; to describe oral health-related behaviour and relate this to variation in oral health parameters; to explore important oral disease determinants in this group using a multivariate approach including relevant biological and socio-behavioural risk indicators; to describe patterns of utilisation of oral health services, including dental attendance patterns, reimbursement of dental treatment (HELFO) and dental tourism.

MATERIALS AND METHODS: The investigation will comprise a self-administered questionnaire and a clinical and radiographic examination of 450 randomly selected 65-year-olds, residing in Oslo. Data collection started in February 2019 and is expected to be completed by the end of the year.

P 10 QUORUM-SENSING MOLECULE DIHYDROXY-2,3-PENTANEDIONE AND ITS ANALOGS AS REGULATORS OF EPITHELIAL INTEGRITY

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BACKGROUND AND OBJECTIVE: The behavior of bacteria within biofilms can be regulated by quorum sensing molecules which at the same time elicit an immune response in host tissues. Our aim was to investigate the regulatory role of dihydroxy-2,3- pentanedione (DPD), the precursor of universal autoinducer-2, and its analogs (ethyl-DPD, butyl-DPD, and isobutyl-DPD) in the integrity of gingival epithelial cells.

MATERIALS AND METHODS: Human gingival keratinocytes were incubated for 24 h with four concentrations (10 μM, 1 μM, 100 nM, and 10 nM) of DPD and its analogs. By using a proliferation kit, the numbers of viable cells were determined. While gelatin zymography used to measure matrix metalloproteinase (MMP)-2 and -9 activities, protein and mRNA expression of occludin detected by western blot and RT-qPCR, respectively.

RESULTS: Incubation of gingival keratinocytes with 100 nM butyl-DPD showed an increase in cell proliferation. MMP-9 activity was elevated when cells incubated with 10 μM of ethyl-DPD, whereas MMP-2 activity did not show any significant change in gingival keratinocytes when incubated without DPD or analogs. Five forms (105, 61, 52.2, 44, and 37 kDa) of occluding have been observed by western blot analyses. Dimeric (105 kDa) occludin forms increased when the cells incubated with 1 μM and 100 nM of DPD and 10 nM of ethyl-DPD, while monomeric (61 kDa) occludin forms increased in the presence of 100 nM isobutyl-DPD. The forms of monomeric (52.2 kDa and 44 kDa) occludin decreased by DPD and ethyl-DPD and enhanced by 100 nM isobutyl-DPD and 10 nM of butyl-DPD, whereas the low molecular weight (37 kDa) occludin forms decreased by ethyl-DPD and elevated in the presence of isobutyl-DPD. The RT-qPCR analysis showed that occludin expression up-regulated when the gingival keratinocytes exposure to 10 μM isobutyl-DPD.

CONCLUSIONS: This study indicates that without affecting the proliferation or gelatinolytic enzyme activities, isobutyl-DPD has the potential to enhance the epithelial integrity by stimulating occludin formation of the exposed cells.

P 11 EVALUATION OF FLOW CHARACTERISTICS OF UPPER AIRWAY BY COMPUTED FLUID DYNAMICS SIMULATION: A CASE STUDY

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BACKGROUND: Computational fluid dynamics (CFD) simulation is a novel method to investigate the airflow behavior for clinical diagnosis and therapy evaluation. The application of CFD in dentistry is relatively new. Some pioneer studies computerized 3D models to investigate the aerodynamic characteristics of patients treated with mandibular advancement device, oral appliance, mandibular setback surgery or rapid maxillary expansion (RME).However, obtaining comparisons between upper airway outcomes among different studies may be complicated and unquantified due to lack of a standardized applied fluid parameter as well as differences in methodological designs.

OBJECTIVE: The purpose of this study was to create CFD model of upper airway applying new parameters for assessing the effects of RME.

MATERIALS AND METHODS: The patient was a 12-year-old boy who presented both the narrowed maxilla and the enlarged adenoid. RME treatment was planned to increase the width of maxillary arch. Two CBCT scans (3D eXam;KaVo, Biberach an der Riss, Germany) were obtained before and after RME treatment. For further analysis, all images are stored in digital imaging and communications in medicine (DICOM) format. The volume of nasal cavity, nasopharynx,oropharynx and laryngopharynx was performed with mimics software. Computational grids of upper airway is created by ICEM CFD 15.0 for performing the fluidic dynamics of the upper airway. The pressure of 0 Pa at the inlet and a flux of 200 mL/s at the outlet were set for the simulation before and after RME with Fluent 15.0. These parameters were analyzed in the two models: static pressure, total pressure (Tp), the pressure drop (∆p), total pressure drop (∆Tp).

RESULTS: The increasing volume of upper airway after RME included nasopharynx 35.6%, oropharynx 38.6% and laryngopharynx 18.4% and the volume of nasal cavity decreased 1.9%. The ∆p of nasal cavity and nasopharynx decreased 9%, 85%, the ∆p of oropharynx and laryngopharynx increased 5%, 332%; The ∆Tp of nasal cavity, nasopharynx, oropharynx and laryngopharynx decreased 6.97%, 66.7%, 69.6% and 18.3% respectively.

CONCLUSIONS: This CFD model of upper airway applied new aerodynamic parameters and indicated that the nasal obstruction caused by enlarged adenoid was reduced after RME treatment.

P 12 HISTOLOGICAL EVALUATION OF HYDROXYAPATITE COATED PEEK IMPLANTS IN AN EXTENDED HEALING MODEL IN RABBIT

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BACKGROUND: Cutting-grinding technique for undecalcified bone was described by Donath and Breuner in 1982 to evaluate bone healing around different alloplastic implants. Polyether ether ketone (PEEK) has excellent mechanical properties close to those of human bone. PEEK as material was introduced in orthopedic surgery to substitute titanium implants mainly in the vertebra.

OBJECTIVE: To evaluate the level of osseointegration after a prolonged healing time of 20 weeks to investigate the delayed effect of the HA coating.

MATERIALS AND METHODS: The samples studied were hydroxyapatite coated PEEK implants or non-coated controls. Forty-eight PEEK implants were inserted in the rabbit femur (n=24) and tibia (n=24) and histologically evaluated at 20 weeks of healing. Twenty-four of the total implants were HA coated (test), and 24 were uncoated PEEK (control).

RESULTS: The mean bone–implant contact (BIC) and bone area (BA) was higher for test compared to control but without statistical significance in femur. No significant difference was shown between the two groups when the three top threads of the implants placed in the tibia were examined. HA coating of implant materials for bone has been found to enhance osseointegration and bone formation in the early stage of healing.

CONCLUSIONS: The hydroxyapatite coated implants were expressed differently over the healing time. From an enhanced new bone formation at implant surfaces after short healing time to matured bone forming at 20 weeks of healing.

P 13 "HELLO, HOW CAN WE HELP YOU?" PRESENTING THE CLINICAL RESEARCH LABORATORY AT UNIVERSITY OF OSLO

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The Clinical Research Laboratory at the University of Oslo Institute of Clinical Dentistry offers both equipment and expertise to support your research projects. We have experience with methods from cell culture to animal models to human samples, including gene expression, confocal microscopy, histology, multiscale x-ray computed tomography, and biomaterial surface analysis. Work can be done on a service or collaboration basis. Here we will give a brief overview of some of the projects we have been involved with, and the instruments available for use. For further information please ask, or see our website: https://www.odont.uio.no/iko/english/about/organization/units/biomaterials/Capacities/ We look forward to working with you!

P 14 IDENTIFICATION OF CYTOKINES AND BONE MARKERS IN APICAL LESIONS FROM PATIENTS WITH PERSISTENT APICAL PERIODONTITIS

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BACKGROUND: Persistent apical periodontitis has been a focus of interest in endodontic research for a long time, with the main focus on microorganisms and their role. However, apical periodontitis is a disease where a complex interaction between the host's immune response and microbial colonization and activity takes place. The genetic events governing the host's reactions to the infection in persistent apical periodontitis is still unclear. Analyses of cytokines in persistent cases of apical periodontitis will help us to gain knowledge about immunological response to apical insults. Proteins are the product of

active genes in the somatic cells during chronic apical inflammatory disease. Therefore, with the use of immunoassays we can acquire information about several aspects regarding protein functions.

OBJECTIVES: The primary object of the project is to acquire insight into the molecular events taking place in persistent apical periodontitis disease and repair. This study tested the hypothesis (H_0) that there is no statistically significant difference between the control and apical samples regarding cytokine expression.

MATERIALS AND METHODS: The research project was approved by the Regional Committees for Medical and Health Research Ethics (REC) REK/biobank approval no 2016/679-3. Signed consent forms were collected from all patients. Samples: Persistent apical lesions from 20 patients with root-filled teeth were removed during apical surgery and stored with no preservatives at -80 degrees centigrade. Crestal healthy bone from 20 patients was collected during surgical removal of healthy impacted third molars and used as control. Protein isolation and detection: Cryocrushing and sonication was performed to induce/complete cell lysis. TRIzol ® reagent was then used for protein isolation and precipitation from the samples. Total protein content was measured using the Thermo Scientific™ NanoDrop™One by the 280 application prior to Luminex due to small sample sizes. MILLIPLEX MAP Human TH17 Magnetic Bead Panel - Immunology Multiplex Assay was used to simultaneous quantify the following analytes in pulverized persistent apical periodontitis-tissue supernatant samples: GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, IL-17F, IL-17E/IL-25, IL-21, IL-22, IL-23, IL-27, IL-28A, IL-31, IL-33, MIP-3α/CCL20, TNF-α and TNFβ. Analytes below the level of detection was excluded. Statistical analysis was carried out using the SigmaPlot software by Systat Software Inc. performing Normality test, and Mann-Whitney Rank Sum Test with 20 controls and 20 samples.

RESULTS: The levels of IL-2 ($P < 0.001$), IL-23 ($P = 0.009$), IL-17E ($P < 0.001$), IL-13 ($P = 0.032$) and TNFalfa ($P = 0.006$) were found to be lower in the apical samples as compared to the control samples. Whereas the levels of IL-6 ($P = 0.019$), IL-21 ($P = 0.022$) and IL-33 ($P = <0.001$) were higher in the apical samples as compared to the control samples.

CONCLUSIONS: The null hypothesis was rejected. Further analysis and research need to be carried out in order to validate the significance of these findings.

P 15 ORAL WELL-BEING IN RADIATED HEAD AND NECK CANCER PATIENTS

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BACKGROUND: Advances in treatment of head and neck cancer (HNC) have led to increased survival. The current study examined some of the common oral side effects after radiotherapy, aiming to get a broader understanding of how these side effects affect oral well-being and quality of life in HNC patients.

OBJECTIVE: To investigate oral well-being in HNC patients after radiotherapy, as compared to age- and sex-matched controls.

MATERIALS AND METHODS: Thirty HNC patients who had completed radiotherapy in the head and neck region (RT-group) (mean age 64±10y), and 30 sex- and age-matched controls (mean age 58±17y) were recruited. All participants underwent a comprehensive oral examination. Decayed, missing, or filled teeth (DMFT) score, secretion of unstimulated whole saliva (UWS, ml/min), oral candida score, and mucous membrane friction test (sliding mirror test: 0= no friction, 1=friction, 2=severe friction) were noted. All participants subjectively evaluated their general and dental health status, on a scale from 0 to 5, where 0 = very poor, and 5 = excellent. A standardized questionnaire was used to assess the existence of burning sensation and foul taste in the mouth or on the tongue, as well as whether oral health issues affected their social life. Intergroup comparisons were tested with Mann-Whitney U test and Person Chi-square using SPSS Version 25. P-values of <0.05 were considered significant.

RESULTS: Compared with the control group, the RT-group demonstrated increased friction of the oral mucous membrane (0.5±0.73 vs 0.0±0.0, $p<0.001$), decreased UWS (0.1±0.09 vs 0.3±0.2, $p<0.001$), fewer number of teeth present (24.0±4.35 vs 26.0±4.1 $p=0.019$), and a higher oral candida score (1.6±1.3 vs 0.7±0.9 $p=0.008$). The RT-group reported worse general health and oral health scores (2.7±0.95 vs 3.3±0.8, $p<0.001$; and 2.7±1.0 vs 3.2±0.7, $p=0.004$, respectively). A higher prevalence of self-reported foul taste in the mouth or on the tongue (26.7% vs 6.7%, $p=0.038$), and a higher prevalence of burning mouth sensation were also found in the RT-group (46.7% vs 0%, $p<0.001$). Furthermore 43.4% patients reported oral dryness to affect their social life vs 0% in the control group ($p<0.001$), while 16.7% of patients reported reduced taste to affect their social life vs 0% of controls ($p=0.044$).

CONCLUSIONS: The post-radiation sequelae of head and neck cancer negatively affected general and oral health, which may in turn influence the patients' social life. An in-depth understanding of the wide array of side effects is important in improving the quality of life in these patients.

P 16 THE EFFECTS OF CHEMORADIATION ON ORAL CARCINOMA PATIENTS CELL VIABILITY USING MYOGEL

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BACKGROUND: The treatment of oral cancer patients includes surgery in combination with radio-, chemo- or targeted therapy. Unfortunately, the survival rate among patients is still low and new treatment approaches are needed. Traditionally, cancer cell lines cultured on a 2-dimentional (2D) surface are used to predict the efficacy of new anti-cancer compounds. However, the current pre-clinical anti-cancer drugs testing has low predicting value for drug efficacy. 3-dimensional (3D) culture systems are increasing to produce more reliable data. However, biologically derived extracellular matrices like Matrigel® differs from human tumor microenvironment and this plays an essential role in cancer drug testing. Our group has developed a human based matrix, Myogel, extracted from human leiomyoma tissue. Myogel is mimicking the in vivo tuomor microenviorment for more reliable in vitro cancer studies.

OBJECTIVES: The aim of our study is to set up a system for testing irradiation and chemotherapy in vitro to predict the efficiency of the cancer treatment.

MATERIALS AND METHODS: For our pilot experiments we used three HNSCC cell lines, one chemotherapy drug Cisplatin and one targeted therapy drug Cetuximab. We used two different concentrations of each drug. Cells where cultured in 2D plastic wells and on top of Matrigel® and Myogel. For irradiating the cells, we used gamma irradiator OB29/4 (STS, Braunschweig, Germany). The irradiation was given as one dose or fractions. The irradiation doses where between 2 and 8 Gy. CellTiter-Glo® Luminescent Cell Viability Assay was used for viability measurement.

CONCLUSIONS/SIGNIFICANCE: By using this method hopefully we are able to predict if irradiation together with cancer drugs is an effective treatment approach for oral cancer patients.

P 17 ENGINEERING OF A THREE DIMENSIONAL BIOSYNTHETIC CONSTRUCT EQUIVALENT TO HUMAN CORNEAL EPITHELIUM AND STROMA

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BACKGROUND: Corneal diseases are the fourth leading causes of blindness and the current treatment is either transplantation of whole cadaveric donor cornea (penetrating keratoplasty, PK) or selective anterior lamellar keratoplasty (ALK) or endothelial keratoplasty (EK). However, corneal transplantation is limited by low availability of donor corneas worldwide. Therefore, bio-engineered sustainable ALK equivalent will be of great clinical interest, as it also contributes to offset the global shortage of donor corneas. Previous studies have tried to culture monolayer human corneal stromal cells (hCSCs) as 2 dimensional (2D) culture methods. However, a 2D culture system can only provide a planar structure of hCSCs, which limits extracellular matrix protein synthesis and leads to loss of expression of specific native hCSC markers such as *KERA*, *CD34*, *ACTA2* and *ALDH3A1*. In addition, successful transplantation of cultured human oral mucosal epithelial cells (hOMECs) represents a 'non-limbal cell approach' to treat limbal stem cells deficiency (LSCD). We hypothesize that hCSCs and hOMECs can be used to reconstruct ALK equivalent tissue graft, which can in future be useful for the treatment of corneal pathologies.

OBJECTIVES: To establish a 3D-tissue construct (ALK equivalent) using *in vitro* expanded hCSCs embedded in biosynthetic scaffold and overlaid with cultured hOMECs.

MATERIALS AND METHODS: Human corneal stroma and oral mucosal biopsies were obtained from cadaveric donors. Monolayer cultured hCSCs were embedded into a biocompatible 3D collagen based scaffold. hOMECs were overlaid on the scaffold-hCSCs composite and cultured further for 2 weeks. Gene and protein expression (α-actin, α-SMA, ABCG2, ALDH1A1, KRT18, OCLN, CD45, CD73, CD90, CD105, CD133) and morphology of the engineered construct was compared with that of native and respective monolayer cultured cells by qRT-PCR, immunocyto/histochemistry and transmission electron microscopy (TEM) assays.

RESULTS: Morphology and expression levels of the markers specific to hCSCs and hOMECs in engineered 3D-construct were similar to the corresponding cells in native cornea.

CONCLUSIONS: These preliminary data show that the engineered 3D-construct using hCSCs and hOMECS is feasible. However, this model needs further optimization and refinement to improve functional and physical characteristics comparable to native cornea.

P 18 C-di-GMP SIGNALING IN TREPONEMA DENTICOLA AND ITS ROLE IN PATHOGENESIS OF PERIODONTAL DISEASES

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BACKGROUND: This project focuses on important clinical challenges in the field of dentistry, namely periodontal diseases and endodontic infections. The periodontal diseases lead to the destruction of the periodontal tissues, while endodontic infections concern the root canal system. Both conditions are tightly linked to an imbalance of oral microbiota. Oral bacteria are mostly associated with biofilm on the teeth (dental plaque), which is known to be more resistant to treatment.

The motile spirochete *Treponema denticola* has been proposed as the key oral pathogen in both clinical conditions. In many bacteria, the second messenger cyclic-di-guanosine monophosphate (c-di-GMP) regulates the biofilm formation, motility and virulence in response to environmental signals. C-di-GMP high intracellular concentration leads to biofilm formation and chronic disease development, while low levels induce motility and acute virulence. The inactivation of a c-di-GMP effector protein (TDE0214) in *T. denticola* indicated that c-di-GMP regulates these phenotypes in this bacterium. This project will characterize the different components of c-di-GMP signaling pathway in more detail.

OBJECTIVE: The objective of this project is to characterize the c-di-GMP signaling network of *T. denticola* and understand its role in pathogenesis.

MATERIALS AND METHODS: This project includes *in vivo*-, *in vitro*-testing and biochemical characterization of proteins involved in c-di-GMP signaling in *T. denticola*. The role and function of the components of the c-di-GMP signaling network will be determined and their involvement in biofilm formation and motility of *T. denticola* will be studied. The *in vitro* approach includes overexpression of proteins of interest from a plasmid in a heterologous host (*E. coli*) and determination of their function through enzyme kinetics and c-di-GMP binding assays. A specific strain of *S. enterica* serovar Typhimurium is used to determine the *in vivo* function of the proteins and their effect on the well-characterized c-di-GMP regulated phenotypes (rdar morphotype and motility).

RESULTS: Twelve *T. denticola* genes were cloned into expression vectors. The expression and purification of the proteins has been started. Preliminary results from biofilm-assays done in *S. enterica* serovar Typhimurium UMR1 show that some of the overexpressed proteins affect the extent of biofilm formation.

CONCLUSIONS: The project is in its early stage, but the preliminary data suggest that unexpectedly few gene-products have shown a change in phenotype in the biofilm-assays. This might indicate that different genes are regulated by environmental conditions, such as oxygen tension specific for the natural environment of *T. denticola*.

P 19 CARIES DETECTION USING A #D INTRAORAL SCANNER EMITTING BLUE LIGHT. AN IN VITRO ASSESSMENT

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BACKGROUND: Caries detection based on fluorescence is one of the most promising technologies for accurate detection of enamel demineralization in early stages. Until recently, this method was only available in 1D or 2D devices. The main limitation of those devices is the challenge in precise comparison of single images/measurements obtained at different periods, which is at large influenced by imaging artefacts and noise. Thus, overcoming this limitation by combining fluorescence with 3D intraoral scans, could potentially improve the applicability of fluorescence, and may reduce the need for using ionizing radiation for caries detection. 3Shape TRIOS A/S was the first to integrate the fluorescence method into a 3D intraoral scanner and manufactured an intraoral scanner emitting both white and blue light. The white light is used for the normal color 3D impression though the blue light is used for the fluorescence method.

OBJECTIVES: (a) to define optimal cut-off limits for detection of occlusal caries lesions using fluorescence induced by the 3D intraoral scanner prototype and (b) to evaluate the validity of the method.

MATERIALS AND METHODS: To define optimal cut-offs, 60 freshly extracted permanent posterior teeth were scanned with a 3D intraoral scanner (prototype based on 3Shape TRIOS 3 intraoral scanner, not commercially available) which emits blue light. A texture representing the fluorescent signal from the tissue was mapped onto 3D models using specific software. Red (R) and Green (G) color components from 250 sound or carious sites located on occlusal surfaces of the teeth were used to calculate a function f(R,G). Histological analysis of the teeth was conducted applying the following scale: D0, sound; D1, enamel lesion; D2, lesion into the 1/3 of dentin; D3, lesion into the 2/3 of dentin and D4, lesion into the 3/3 of dentin.

RESULTS: For Sensitivity (SE) – Specificity (SP) sum above 1.6, three optimal cut-offs for the f(R,G) were defined corresponding to histological levels D1, D2 and D3. To assess the method's validity, the defined optimal cut-offs were applied on a new set of 48 teeth (200 sound or carious sites on occlusal surfaces). SE, SP and the area under the ROC curve (Az) were calculated based on histological analysis: D1: SE 0.88, SP 0.9, Az 0.94; D2: SE 1, SP 0.76, Az 0.91; D3: SE 1, SP 0.83, Az 0.87.

CONCLUSIONS: Caries detection using the 3D intraoral scanner emitting blue light showed good *in vitro* performance with high SE, SP and Az values for the three defined optimal cut-offs. Further studies with larger sample size are needed to optimize and validate the defined cut-offs.

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P 20 FULLY HUMAN TUMOUR-BASED MATRIX IN THREE-DIMENSIONAL SPHEROID INVASION ASSAY

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BACKGROUND: Tumor microenvironment is an essential element of cancer progression. 2D cell culture-based assays are commonly used in *in vitro* cancer research. However, they lack several basic elements that form the tumor microenvironment. Therefore to study carcinoma progression, 3D assays with biologically relevant matrix is needed. Multicellular tumor spheroids are widely used 3D assay to study cancer cell invasion, as they mimic several features of the *in vivo* condition. Currently, animal-derived matrices (e.g. mouse sarcoma-derived Matrigel and rat tail type I collagen) are mainly used in the spheroid assay and none of the commercially available matrices are originated from human tumor tissue. Taking into consideration the differences between the human tumor microenvironment and animal-derived matrices, we developed a human leiomyoma-derived matrix (Myogel).

OBJECTIVES: In this study, we aimed to develop a fully human tumor-based 3D spheroid invasion assay to study cancer cell invasion. The assay is supported by computer-based invasion analysis.

MATERIALS AND METHODS: Multicellular tumor spheroids were generated by culturing the carcinoma cells in an ultra-low attachment 96-well round bottom plate for four days. The 3D invasion assay was done by adding Myogel/fibrin gel in to the wells. The gel consisted of Myogel, thrombin, aprotinin and fibrinogen. Matrigel and type 1 rat tail collagen were also used for comparison purposes. The gel was added to the wells and after 30 min when the gel was completely solidified, 100 ul of media was added on top of the gel. Spheroids were imaged daily using an inverted microscope and the images were analyzed with ilastik and ImageJ.

RESULTS: Cells in the Myogel/fibrin matrix invaded rapidly after one day and extended into the matrix as strands. Cells in the Matrigel did not invade into the surrounding matrix, instead they formed an asymmetrical structure. Cells in the collagen invaded slightly, but due to the matrix shrinkage the analysis was difficult.

CONCLUSIONS: Replacing 2D with 3D cell culture assays provides more accurate information on cancer cell behavior. The presented method provides the first fully human tumor-based 3D assay to evaluate the invasiveness of cancer cells within human tumor microenvironment mimicking matrix. The assay is also more ethical than using Matrigel since Myogel is extracted from the leftover material of human leiomyoma tumor.

P 21 BIOMIMETIC NANOCELLULOSE-NANOHYDROXYAPATITE SCAFFOLDS FOR BONE TISSUE ENGINEERING

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BACKGROUND: Bone is a biocomposite of nanohydroxyapatite (nHA) embedded in a gel-like matrix of collagen and polysaccharides. In analogy with collagen, wood-based cellulose nanofiber (CNF) is the main building block of trees. CNF is animal and microbial-origin-free polysaccharide

biopolymer that replaces collagen due to its availability, biocompatibility and low cost. Therefore, the aim of this study was to prepare and characterize biomimetic porous scaffolds for bone tissue engineering by addition of different concentrations of nHA to CNF matrix.

MATERIALS AND METHODS: CNF hydrogel was mixed with nHA in four ratios (CNF:nHA = 1:0, 1:1, 2:1 and 4:1 W/W). The mixtures were then cast into 96-well plates, frozen at -20 °C and finally freeze-dried. Scaffolds were analysed with a micro-CT to determine the porosity of the scaffolds. Osteoblast-like cells (Saos-2) were then seeded on the scaffolds (1×10^5 cells/scaffold). The seeding efficiency was quantified by Alamar Blue assay after 4 hours. Cell viability was assessed with Live/Dead staining. Cell proliferation was measured with PicoGreen assay up to 14 days. Early osteogenic differentiation was evaluated by ALP-kit.

RESULTS: The highest concentration of nHA (1:1) demonstrated the smallest pore size with least variation whereas the lowest concentration of nHA (4:1) revealed largest pore size distribution. The seeding efficiency experiment demonstrated that the (4:1) group had the highest initial cell number. All groups maintained high cell viability over 4 days indicating the nontoxic effect of the scaffolds. Moreover, all scaffolds supported cell proliferation and the highest value was observed with the (4:1) group on day 14. The scaffolds supported cell function as indicated by the continuous production of ALP throughout the culture period.

CONCLUSIONS: The addition of nHA to CNF matrix successfully produced biomimetic scaffolds with tailorable physical and biological properties. Composite scaffolds supported osteoblastic cell proliferation and differentiation without cytotoxicity. The combination of nHA and CNF may be a promising scaffold for bone tissue regeneration.

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P 22 POOR ORAL HEALTH ASSOCIATES WITH METABOLIC SYNDROME

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BACKGROUND: Poor oral health such as periodontal disease associates with overweight, metabolic diseases, particularly type 2 diabetes (T2D), and cardiovascular diseases. An association has also been found between periodontitis and metabolic syndrome (MetS) which is the background for this study.

OBJECTIVES: To study the association of oral health with MetS in women with a history of high risk for gestational diabetes (GDM). Our hypothesis was that women with good oral health would have less MetS.

MATERIALS AND METHODS: Women at high risk for GDM participated in a lifestyle intervention trial in 2008–2012 (n=720). 5-year post-partum an oral health examination was performed in 115 women out of 348 available at the 5-year follow-up. Number of teeth, total dental index (TDI) and diseased, missing, filled teeth (DMFT) index were calculated. Bleeding on probing (BOP), probing depth (PD), visible plaque index (VPI), and clinical attachment level (CAL) were recorded. Periodontal inflammatory burden index (PIBI) was calculated. Panoramic radiographs were taken and

signs of infections documented. Information on oral health habits, symptoms and subjects' self-assessed oral health status were collected with a questionnaire.

RESULTS: Of the women, 45% had a history of GDM in the index pregnancy and 20.5% (n=23) were diagnosed with MetS 5 years post-partum; 4% (n=4) had diabetes mellitus, respectively. Women with MetS had higher BOP-index score (52.2% *vs.* 44.3%, p=0.011). The association was not explained by differences in oral health habits, which were similar in the groups. There was a trend towards poorer periodontal health in women with MetS but the difference between groups was not significant: PIBI (19% *vs.*14%, ns.), VPI (19% *vs.*14%, ns.), TDI (1.96 *vs.* 1.65, ns.) and periodontitis (no periodontitis or mild periodontitis 61% *vs.* 75%; moderate or severe periodontitis 39% *vs.* 24%, ns.).

CONCLUSIONS: Our hypothesis was partly confirmed by showing that high BOP as a sign of early-stage periodontal disease was independently associated with MetS. However, no difference between groups was found in the prevalence of periodontitis.

P 23 THE ASSOCIATION BETWEEN SUBSTANCE USE DISORDER AND ORAL HEALTH THROUGH ASI AND DENTAL EXAMINATION AMONG A GROUP OF INDIVIDUALS LIVING IN STOCKHOLM, SWEDEN

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BACKGROUND: Validated self-reporting questionnaires such as Addiction Severity Index (ASI), have been widely used. However, to our knowledge, no previous studies have used ASI as a screening instrument among patients of the general dental practice in Sweden.

OBJECTIVES: This cross-sectional study describes the current oral health among a group with substance use disorders and their addiction habits. This was done with the help of the validated screening tool, ASI and a clinical dental examination.

MATERIALS AND METHODS: During this study the Swedish version of ASI has been used in connection with a clinical dental examination. The questionnaire was done face-face in a dental clinic or at one of the addiction treatment centers in Stockholm's County. The dental examination was done in a dental clinic.

RESULTS: Of the 102 participants, n: 84 (82%) were male and n: 37 (36%) were homeless. A total of n: 96 (94%) of the participants answered the ASI questionnaire and it was found that n: 36 (54%) took the substance orally and n: 28 (42%) injected the drug intravenously. Participants were divided according to their substance use disorder in four different groups; alcohol 29% (n: 30), Cannabis 28% (n: 29), Central Nervous System Stimulants (CNSS) 21% (n: 21); Opiate 22% (n: 22). The mean (sd) number of teeth, DMFT and periodontal pockets > 6 mm where poorer among those who injected the substance intravenously (CNSS and opiate) 17.54(9.49), 18.68 (7.32), 3.39 (5.92) vs those who took the substance orally (alcohol) 22.26 (7.27), 14.57 (6.98), and 7.74 (18.30). The results from the ASI also revealed that among the participants, the opiate group was the group which had experienced most problematic day´s with their substance use disorder during the past 30 days period compared to the alcohol, cannabis and CNSS groups.

CONCLUSIONS: These results indicate that the CNSS and the opiate groups have higher scores in the ASI's seven different domains and also in conjunction with the dental examination. It seems that poor oral health is more common among individuals that injects the substance intravenously (CNSS, opiate).

P 24 EFFECT OF HUMAN PLATELET LYSATE ON RAT MESENCHYMAL STEM CELLS FOR BONE REGENERATION – AN *IN VITRO* STUDY

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BACKGROUND: Bone marrow mesenchymal stem cells (BMMSCs) are one of the most promising sources of cell therapy for bone regeneration. The use of human platelet lysate (hPL) over fetal bovine serum (FBS) as a culture supplement during human BM-MSC expansion has been studied extensively and is clearly preferred for clinical translation since FBS is xenogeneic, poorly characterized and forms potential immunogenic source in cell therapies. Rat BMMSCs, usually cultured in FBS, are frequently used for in vitro testing of MSC strategies. However, little is known about the effects of hPL on rat BM-MSCs as a clinically relevant alternative for pre-clinical rodent models.

OBJECTIVES: To characterize and compare the *in vitro* properties, such as MSC-yield, growth kinetics, immunophenotype and multi-lineage differentiation potential, of rat BM-MSCs isolated and expanded in hPL- versus FBS-supplemented culture medium.

MATERIALS AND METHODS: A commercial locally-manufactured hPL (Bergenlys®, Bergen, Norway) was used in this study. Following ethical approval, rat BMMSCs were isolated in either 10% PL- or 10% FBS-supplemented medium. Both types of BMSCs , expanded and passaged separately, were evaluated for their morphology, growth rate [population doubling time (PDT)], viability, colony forming unit-potential (CFU), immunophenotype (via Flow cytometry) and tri-lineage (osteogenic, adipogenic and chondrogenic) differentiation capacity Expression of osteogenic specific genes was evaluated by real time PCR.

RESULTS*: Rat BM.MSCs grown in hPL have a very distinct (highly elongated) morphology as compared to smaller FBS cultured cells. PDT was found to be slightly higher in FBS up to passage 3, while CFU was significantly higher in FBS cultured cells.

CONCLUSIONS*: Preliminary results suggests that hPL markedly reduced proliferation and colony formation in rat BMMSCs leading to very slow growth after passage 3 as compared to FBS. This could be attributed to the interspecies variability between the hPL and rat MScs. Future studies should evaluate the efficacy of rat-derived PL for expansion of rat BMMSCs in comparison to FBS.

*NOTE: Some experiments are yet to be analyzed. Complete results and conclusions will be available in time for the meeting.

P 25 PRO- OR ANTI-INFLAMMATORY EFFECTS OF CURCUMIN NANOPARTICLES ON OSTEOARTHRITIS TREATMENT?

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BACKGROUND: Osteoarthritis (OA) is a major cause of severe joint pain, and physical disability in the aging population across the world. This disease has traditionally been defined as a prototypical non-inflammatory arthropathy, but recently there has been convincing evidence to support that it has an inflammatory phase. Thus, several of biomedical scientists and surgeons have currently focused on using anti-inflammatory mediators for treating this disease. Over the past few years, curcumin nanoparticles have gained much attention among biomedical scientists as potential anti-inflammatory mediators for treating OA.

OBJECTIVES: In this study, some thermosensitive chitosan-based hydrogels were designed as delivery systems for curcumin nanoparticles (as an anti-inflammatory agent with natural origin) to *in vivo* study their anti-inflammatory capacity.

MATERIALS AND METHODS: An osteochondral defect of 4 mm in diameter and depth was made by mechanical drilling in the femoral center of the trochlea in 18 New Zealand White rabbits. Previously examined from an *in vitro* study, chitosan-based hydrogels containing 12% βeta-glycerolphosphate (β-GP) were synthesized to load curcumin nanoparticles. Three groups including: hydrogels without drug, hydrogels containing nanocurcumin, and control groups were considered. During the whole study, rabbits were clinically examined and after 4 or 12 weeks, the rabbits were sacrificed. Joints were evaluated by using histological evaluations.

RESULTS: Results from the histological evaluation of *in vivo* study showed that, however, chitosan-based hydrogels could potentially improve cartilage regeneration, the implantation of curcumin nanoparticles were not able to enhance cartilage repair even in comparison with the control group in both time points.

CONCLUSIONS: More in vitro& in vivo inflammatory studies should be done on the pro- and/or anti-inflammatory effects of curcumin nanoparticles on the cartilage regeneration.

P 26 3D PRINTED GELATIN-GENIPIN SCAFFOLDS FOR TEMPOROMANDIBULAR CARTILAGE REGENERATION

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BACKGROUND: Degenerative joint diseases cause destruction of bone and cartilage in the temporomandibular joint (TMJ) resulting in pain and limited jaw movement. Limitations of current treatments increased the interest in regenerative strategies by combining stem cells and implantable biomaterials (scaffolds). Among biomaterials, gelatin is an ideal biomimetic scaffold for cartilage regeneration. However, to fabricate gelatin-based scaffolds, several issues must be considered including biocompatible crosslinking mechanism, precise control of the scaffold structure, degradation rate, and mechanical properties.

OBJECTIVES: To address the following challenges of gelatin scaffolds for cartilage regeneration. 1) To utilize genipin, an organic compound derived from gardenia plants, as a cell-friendly crosslinking agent. 2) To control the outer shape and inner pore structure of the scaffolds utilizing 3D printing technology. 3) To evaluate the feasibility of the printed scaffolds for cartilage regeneration utilizing human bone marrow mesenchymal stem cells (hBMSCs).

MATERIALS AND METHODS: Gelatin from porcine skin was mixed with distilled water (10% w/v) at 40ºC. The solution was poured into a printing cartridge and printed on a cooled platform (4ºC). The structures had a design of a square composed of 16 perpendicular layers with 1.5 mm strand distance and a shift of 0.75 mm every third and fourth layer. The printed structures were then crosslinked in aqueous genipin solution (1% w/v) for 48 h and freeze-dried for 48 h. The structural characterization was evaluated by optical microscope and micro-CT. The mass change of the scaffolds was evaluated over 28 days in phosphate buffered saline (PBS). The Young's modulus of the scaffolds was calculated from compression tests before and after crosslinking. Furthermore, attachment, viability, proliferation and chondrogenic differentiation of hBMSCs were evaluated by methods of live/dead stain, scanning electron microscope, DNA quantification assay and PCR in comparison to cell pellets as controls.

RESULTS: The printed scaffolds had good structural fidelity and precision. After crosslinking, scaffolds showed high elasticity and good stability in PBS. Biologically, genipin had no cytotoxic effect. The scaffolds supported cell attachment and proliferation over 7 days. Although, the cell pellets demonstrated higher expression of the chondrogenic related genes including SOX9, Aggrecan, Collagen type 1 (Col 1), Col 2, the scaffolds downregulated the expression of the hypertrophy marker of Col 10, which is expressed when cells undergo endochondral ossification.

CONCLUSIONS: 3D printed gelatin-genipin scaffolds demonstrate high elasticity, maintain good cell viability, support chondrogenic differentiation and therefore have great potential for TMJ cartilage regeneration.

P 27 LIGHT ATTENUATION THROUGH DIFFERENT SHADES AND THICKNESSES OF CAD/CAM MATERIALS

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BACKGROUND: Inadequate energy delivery to a photo-curable resin restoration affects both its durability and properties. Successful delivery of light energy to the photo-cured or dual-cured cement is dependent on many factors; the light curing unit, the translucency, the thickness and the type of restoration.

OBJECTIVE: The aim of this study was to assess the influence of thickness and shade on the light intensity transmitted through a CAD/CAM material.

MATERIALS AND METHODS: Six study groups; four groups of Tetric® CAD; HT A2, MT A2, MT A3, MT A3.5 and two groups of GC InitialTM LRF CEREC; A3 HT and A2 LT were used. Each group included seven specimens of different thicknesses; 0.3±0.02 mm, 0.5±0.02 mm, 1±0.02 mm, 1.5±0.02 mm, 2±0.02 mm, 2.5±0.02 mm, 3±0.02 mm. The disc were sectioned from CAD/CAM blocks and polished. Elipar™ DeepCure-S (3M ESPE) LED Curing Light of maximum irradiance 2098 mW/cm2 was used. The intensity of the light output was evaluated using Bluelight Analytics – MARC LC before testing the prepared discs using the same device. Thereafter, light intensity transmitted through the discs was evaluated by placing the disc on the selected aperture. Five readings were recorded for each disc with the irradiation centered on the disc surface for 10 seconds. The beam profiles of the samples were also evaluated for different shades and thicknesses. Data were statistically analyzed using two-way ANOVA ($p=0.05$) and Tukey's test.

RESULTS: The light transmitted through the specimens decreases to a minimum of 85 mW/cm2 through a 3mm thick restoration and is inversely proportional to the thickness. Light transmission is attenuated significantly differently for HT and LT versions and for different shades of the materials ($p<0.05$).

CONCLUSIONS: Even a relatively thin layer of restoration decreases the light intensity significantly, therefore, the light curing time should be adapted during cementation to compensate for decreased light intensity.

P 28 DEFINING THE ROLE OF PORPHYROMONAS GINGIVALIS PEPTIDYL ARGININIE DEIMINASE (PPAD) IN ORAL CARCINOMA

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BACKGROUND: *Porphyromonas gingivalis* is an opportunistic microorganism of the oral microbiota well-known for its involvement in periodontal disease. In addition to periodontitis, evidence points to a role for *P. gingivalis* in the development of chronic diseases such as diabetes, cardiovascular diseases, rheumatoid arthritis and several orodigestive cancers. The ability of *P. gingivalis* to cause

these diseases is determined by its arsenal of virulence factors including fimbriae, lipopolysaccharides, gingipains, hemolysins and peptidyl arginine deiminase (PPAD). The latter is an enzyme unique for *P. gingivalis* that converts arginine residues in host proteins into citrulline, a non-genetically coded amino acid. Interestingly, recent studies show that PPAD can citrullinate histone H3, one of the core histone proteins present in the nucleosome that packages DNA into chromatin. Histone citrullination affects gene transcription and chromosome decondensation. Moreover, *P. gingivalis'* contribution to cancer development has been linked to global changes in the epigenetic profile of host cells, including changes concerning factors responsible for epigenetic regulation of chromatin. Therefore, further investigation is needed to fully understand epigenetic events that occur during *P. gingivalis* infection, and to discover whether virulence factors that bind and modify chromatin can affect expression of significant genes involved in cancer progression.

OBJECTIVES: To examine the ability of peptidyl arginine deiminase (PPAD) from *P. gingivalis* to reorganize host chromatin at specific loci important in cell proliferation and survival.

MATERIALS AND METHODS: Full-length and truncated versions of PPAD tagged with either HA or EGFP were constructed in eukaryotic expression vectors. Oral squamous cell carcinoma cell line PE/CA-PJ49 was transfected with established constructs and expression of PPAD was investigated by immunobloting. Distribution of PPAD enzyme was analyzed by laser scanning confocal microscopy. HA-tagged PPAD constructs will be expressed in 3D tissues constructed with gingival keratinocytes and fibroblasts for use in chromatin immunoprecipitation (ChIP) experiments.

RESULTS: Low levels of PPAD-EGFP expression were obtained in PE/CA-PJ49 cells. Fluorescence microscopy analysis showed that PPAD was present in the whole cell interior with an accumulation in the nucleus.

CONCLUSIONS: Our preliminary results support so far a role of PPAD in epigenetic modification of host cells following *P. gingivalis* infection. Ongoing experimental work should ascertain the nature of these epigenetic modifications and the identity of the genetic loci affected.

P 29 NOVEL ASPECTS IN INFLAMMATION-MEDIATED ORAL CARCINOGENESIS

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BACKGROUND: Oral lichen planus (OLP) is a chronic inflammatory disease of oral mucosa, which is categorized as an oral potentially malignant disorder (OPMD). Oral cancer commonly affects the mobile tongue as oral tongue squamous cell carcinoma (OTSCC), which has dismal prognosis. Histamine (Ha) signals via four G protein-coupled histamine receptors (H1R-H4R). Classical Ha-medications are ineffective in treating OLP or OTSCC patients. The discovery of H4R has paved the way for novel perspectives in histamine research by modulating the inflammatory responses. We therefore investigated the interplay between H4R and inflammatory mediator signalling in the pathogenesis of OLP and OTSCC.

MATERIALS AND METHODS: Tissue samples from OLP, oral epithelial dysplasia (OED) and OTSCC patients, and healthy controls were utilized. The in vitro experiments were performed on normal human oral keratinocytes (HOKs), OTSCC cells (HSC-3 and SCC-25), and human mast cells (MCs). For in vitro internalization and functional assays, two specific H4R ligands (agonist HST-10,

and inverse agonist ST-1007) were used. Protein expression of histamine receptors, transporters and metabolizing enzymes, and other antigens were assessed in tissue samples and cell lines by immunostaining. Gene levels were quantified by qRT-PCR and the highly-sensitive droplet-digital PCR technology. Western blotting, flow cytometry, and high-performance liquid chromatography were also used.

RESULTS: H4R is expressed in HOKs that fully internalize H4R-ligands in clathrin-dependent manner. In contrast, OLP, OED and OTSCC samples exhibited lower levels of H4R, which was negatively correlated with MC-count and OTSCC-grade. HOKs are also fully equipped with Ha-synthesizing, transporting and degrading molecules. Interestingly, Ha-synthesizing enzyme was highly induced in OLP patients while Ha-degrading enzymes were inhibited. HOKs showed dose-dependent Lipopolysaccharides (LPS)-driven release of Ha, which interfered with epithelial adhesion molecules (integrins). We next showed that toll-like receptors (TLRs) are essential players in OLP. TLRs were upregulated in OLP lesions, particularly for TLR4, which is necessary for alarmin signalling including LPS. Importantly, LPS and MC-mediators regulated several oral oncogenes, while H4R-stimulated cells revealed a marked resistance to apoptosis. Furthermore, LPS and Ha influenced human beta defensin 2 (hBD-2) expression, which was highly induced in OLP. Unexpectedly, hBD-2 protein was subsided in OTSCC tissues with a marked downregulation of its transcript in cancer cells. Histamine synergistically induced TNF-α- and IFN-γ-mediated hBD-2 production in HOKs. Interestingly, targeting H4R seems to regulate TNFα- and LPS-mediated expression of hBD-2.

CONCLUSIONS: Human oral epithelial cells are "non-professional" histamine producing cells—capable to synthesize, release, and degrade low levels of endogenic histamine. High levels of histamine may downregulate H4R as well as key integrity molecules in HOKs and may enhance subsequent bacterial invasion in OLP. In this regard, our findings suggest a potential role of alarmin-mediated response in OLP pathogenesis, by mediating LPS signalling and enhancing further immune response and histamine production. In addition, our results indicate that histamine/H4R crosstalk signalling with LPS and MCs could in part be involved in OLP and the potential inflammation-driven tumorigenesis. This was further supported by the ability of H4R to regulate cell apoptosis and modulate antibacterial response in HOKs.

P 30 EVALUATION OF CELL RESPONSES TOWARDS DMSO-SOLVATED EXPERIMENTAL ADHESIVES

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BACKGROUND: Despite substantial advances in resin-dentin bonding over the past decades, reduction in bonding effectiveness of currently available dental adhesives remains a major limitation in modern adhesive dentistry. One successful approach to improve the long-term bonding performance of relatively hydrophilic resins is the partial substitution of commonly used organic solvents by dimethyl sulfoxide (DMSO; $(CH_3)_2SO$). However, there are no studies where the relationship among the presence of DMSO, degree of resin hydrophilicity and physico/mechanical properties of commonly used BisGMA-TEGDMA-HEMA bonding resins have been investigated together to better understand the effect DMSO on polymer network formation and durability.

OBJECTIVES: To evaluate the cytotoxicity of experimental resins with various concentrations of DMSO in a dentin barrier test simulating the clinical circumstances.

MATERIALS AND METHODS: Several ascending concentrations of DMSO (0, 0.01, 0.1, 1, 5, and 10 w/w %) were used to solvate a hydrophobic (R2: 70% BisGMA, 28,75% TEGDMA, 0,25% CQ, 1% EDMAB) and hydrophilic (R5: 40% BisGMA, 28,75% HEMA, 30% 2MP, 0,25% CQ) experimental methacrylate-based resins. Three dimensional cultures of SV40 transfected pulp derived cells (Thonemann and Schmalz, Arch Oral Biol, 45, 857-869, 2000) were transferred into an in vitro dentin barrier test device with dentin slices of 400 µm thickness (n=8/experimental group). After 24 h incubation at 37°C, the solvated experimental adhesives were applied into the cavity part of the device for 10 s and light cured for 10 sec. A glass ionomer positive control and non-toxic polyvinylsiloxane negative control (100% cell vitality) were used as control groups. Cell viability after exposure to the bonding agents was determined by dimethylthiazolediphenyltetrazolium bromide (MTT). Data were analyzed by ANOVA and Tukey test (α=0.05).

RESULTS: The cell viability of experimental test groups were not significantly different compared to negative control ($p>0.05$) but was significantly higher than positive control ($p<0.05$). Incorporation of 5-10% DMSO to R2 or R5 showed a slight reduction in cell viability compared to lower concentrations ($p>0.05$).

CONCLUSIONS: With the residual dentin barriers of 400 µm, the incorporation of DMSO to hydrophilic or hydrophobic methacrylate resins did not show any cytotoxic effects on transfected pulp derived cells.

P 31 SOL-GEL DERIVED BIOACTIVE COATINGS ON ZIRCONIA ENHANCE BIOMECHANICS OF SOFT-TISSUE ATTACHMENT

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OBJECTIVES: The purpose of this study was to evaluate the mechanical properties of the attachment apparatus between gingival tissue and TiO_2 coated zirconia, aimed to be used as an implant abutment material.

MATERIALS AND METHODS: Experimental cylindrical zirconia implants (2 mm diameter x 10 mm) were used in two experimental groups (n=4) with sol-gel derived TiO_2 coated and uncoated (control). Full thickness gingival explants were dissected from mandibles of freshly slaughtered pigs using a 6 mm biopsy punch and then rinsed in PBS supplemented with penicillin, streptomycin and amphotericin B. Each of the implants was autoclaved for 20 min at 121ºC and then inserted into the centre of the explants, following placement of the specimens individually at an air/liquid interface on a stainless steel grid, in wells containing Eagle's minimum essential medium supplemented with antibiotics and essential amino acids. The specimens were incubated at 37ºC in a 5 % CO_2 environment with the culture medium changed every 24 h up to 7 and 14 days in culture. The dynamic modulus and creeping modulus of the interface between the gingival tissue and the implants in shear mode were measured using a novel technique in dynamic mechanical analyser (DMA 242E Artemis, Netzsch).

RESULTS: Coated zirconia specimens showed substantially higher dynamic modulus under physiological conditions (30 µm amplitude at 1 Hz) compared to uncoated control at both days 7 (+88% vs. control) and 14 (+109%). Under creeping conditions (pseudo-static) the modulus of adhesion was also improved for the coated specimens at both days 7 (+5%) and 14 (+55%).

CONCLUSIONS: Sol-gel derived TiO_2 coatings on zirconia were proven to enhance soft tissue attachment, forming a stronger adhesion between the gingival tissue in contact with TiO_2 coatings especially under physiological dynamic loading.

P 32 ADENOVIRUS MEDIATED GENE DELIVERY OF BMP2 ALONE IS SUPERIOR TO COMBINED DELIVERY OF BMP2 AND VEGFA IN HEALING OF CRITICAL-SIZED RAT CALVARIAL BONE DEFECT

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BACKGROUND: Bone tissue engineering (BTE) is a promising alternative to autologous bone grafts and bone graft substitutes. Selection of appropriate osteoinductive and/or angiogenic growth factors, a suitable delivery method and a proper supportive scaffold are critical for a successful outcome in BTE. Apart from osteogenesis, angiogenesis of the tissue engineered bone is crucial for its survival and integration to the defect area for successful bone regeneration. Accordingly, several studies have employed the combined delivery of BMP2 (osteogenic factor) and VEGFA (angiogenic factor) to promote angiogenesis and bone regeneration. Nevertheless, the outcomes of these studies are quite inconsistent.

OBJECTIVES: Utilizing adenovirus mediated gene expression strategy in bone marrow stromal cells (BMSC) seeded onto a porous poly(LLA-co-CL) scaffold, the study aimed to investigate the angiogenic and osteogenic abilities of BMP2 and VEGFA administered alone or in combination.

MATERIALS AND METHODS: Human BMSC were engineered to express BMP2 alone or in combination with VEGFA and by utilizing adenoviral vectors, were seeded onto poly(LLA-co-CL) scaffolds. Changes in angiogenic and osteogenic gene and protein levels and *in vitro* mineralization were examined by using quantitative RT-PCR, alkaline phosphatase assay and alizarin red staining. Furthermore, *in vivo* critical-size rat calvaria defect model was used to investigate the effect on angiogenesis and osteogenesis of BMP2 alone or in combination with VEGFA by using micro computed tomography (µCT), histology and quantitative RT-PCR.

RESULTS: Both mono delivery of BMP2 and the combined delivery of a lower ratio of VEGFA and BMP2 (1:4) led to up-regulation of osteogenic genes (*Alpl* and *Runx2*) and increased calcium deposition *in vitro*, compared to controls. Micro computed tomography (microCT) analysis of the rat calvarial defect at 8 weeks showed that mono delivery of BMP2 (43.37± 3.55 % defect closure) was the most effective in healing calvarial bone defect, followed by the combined delivery of BMP2 and VEGFA (27.86 ± 2.89 %) and other controls. Histological and molecular analyses supported the microCT findings. Analysis of the angiogenesis, however, showed that both mono delivery of BMP2 and combined delivery of BMP2 and VEGFA had similar angiogenic effect.

CONCLUSION: Overall, the results showed that the mono delivery of BMP2 was more effective in comparison to the combined delivery of BMP2 and VEGFA in bone regeneration at the critical-size rat calvarial bone defect.

P 33 FRACTOGRAPHIC ANALYSIS OF CLINICALLY FAILED MONOLITHIC AND VENEERED ZIRCONIA CROWNS

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BACKGROUND: One of the main causes for clinical failure of zirconia crowns is fracture of the crowns. Several factors such as material choice, crown design and load distribution affect the clinical survival. A fractographic analysis of the fracture surface is a qualitative method to map the fracture pattern. This method is the first step in understanding the mechanisms of clinical failure. With additional clinical information it might be possible to minimize adverse factors and thus improve the clinical survival of the crowns.

OBJECTIVES: The aim of this study is to assess the fracture pattern of clinically failed zirconia dental crowns.

MATERIALS AND METHODS: Thirty-three fractured zirconia crowns (15 monolithic, 18 veneered) were collected from general dental practices. The material comprised of incisors, premolar and molar crowns. The clinical time until fracture ranged from few months to several years. The crowns were ultrasonically cleaned first in an enzymatic solution then in NaOCl, rinsed with distilled water, ethanol and air dried. All crowns were examined by optical microscopy for overall fracture patterns. Some crowns were examined with scanning electron microscopy for detailed mapping and determination of the fracture origin.

RESULTS: Three types of fracture patterns were identified. Type 1: starting point at the crown margin (n = 21). Type 2: at the inner axial wall (n = 3). Type 3: at the occlusal intaglio surface (n = 2). Fracture origins at the inner axial wall were observed only in the monolithic crowns. Most of the crowns in both the veneered (n=13) and monolithic (n=8) crowns had fracture start at the crown margin. Six crowns were excluded due to missing parts that could have identified the starting points.

CONCLUSIONS: Most of the crowns had starting point at the crown margin. This indicates that crown margin is an area exposed to increased stress concentration regardless of crown type and design. Nevertheless, the study shows that zirconia crowns can fracture in several different ways, which indicates that determining the underlying cause of clinical failure is complicated and multifactorial. Thus continuous retrieval analyses are useful for detecting failure modes of dental zirconia crowns.

P 34 IMPACT OF NARROW SPECTRUM PENICILLIN V ON THE ORAL AND FECAL RESISTOME IN A CHILD TREATED FOR ACUTE OTITIS MEDIA

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BACKGROUND: Antibiotic overuse has led to a global emergence of resistant bacteria, and children are among the most frequent users. Most studies with broad-spectrum antibiotics show severe impact on the resistome development of patients. Although narrow-spectrum antibiotics are believed to have less side effects, and are therefore recommended, their impact on the microbiome and resistome is mostly unknown.

OBJECTIVES: The aim of this study was to investigate the impact of the narrow-spectrum antibiotic phenoxymethylpenicillin (Penicillin V) on the microbiome and resistome of a young child treated for acute otitis media (OM).

MATERIALS AND METHODS: Oral and fecal samples were collected from a one-year child before (day 0), during (day 5) and after (day 30) receiving Penicillin V against OM. Metagenomic sequencing data was analyzed to determine taxonomic profiling, using Kraken and Bracken software, and resistance profiling, using KMA in combination with the ResFinder database.

RESULTS: In the oral samples, 11 antimicrobial resistance genes (ARGs), belonging to four different classes, were identified at baseline. At day 5, the abundance of some ARGs were increased, some remained unchanged, while others disappeared. At day 30, most ARGs had returned to baseline levels, or lower. In the fecal samples, we observed seven ARGs at baseline and five at day 5, with only one gene observed at day 5 being present at baseline. At day 30, the number of ARGs increased to 21 ARGs from seven different classes.

CONCLUSIONS: Penicillin V had a remarkable impact on the fecal resistome indicating that even narrow-spectrum antibiotics may have important consequences in selecting for a more resistant microbiome.

P 35 CHANGES IN PROTEIN PROFILE IN BONE MARROW EXTRACTS BEFORE AND ONE YEAR AFTER GASTRIC BYPASS SURGERY

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BACKGROUND AND OBJECTIVE: The long-term skeletal changes after bariatric surgery are largely unknown, and identifying molecular mechanisms related to reduced bone health after long-term weight loss may reveal novel approaches for reducing the burden of these changes. For better knowledge about bone status, we aimed to identify changes in the protein profile in bone marrow aspirates after weight loss due to gastric bypass surgical intervention.

MATERIALS AND METHODS: Proteins in bone marrow aspirates from 9 patients before and 1 year after gastric bypass surgery (2013/1159/REK Sør-Øst B), were isolated using Trizol (Thermo Fisher Scientific). Total protein content was identified using the BCA Assay (Thermo Scientific). The amounts of specific proteins, such as cytokines (HCYP2MAG-62K;Millipore Merck) and bone markers (HBNMAG-51K) in various samples, were determined by the Luminex 200 system where acquired fluorescence data were analyzed using the 3.1 xPONENT software (Luminex).

RESULTS: Bariatric surgery resulted in reduced bone mineral density ($p = 0.018$) and enhanced levels of osteocalcin ($p=0.001$). In the bone marrow aspirates a reduction in the level of vascular endothelial growth factor (VEGF) ($p=0.046$) and enhanced levels of osteopontin (OPN) ($p=0.021$) and interleukin-1β (IL-1β) ($p = 0.025$) were observed.

CONCLUSION: The bone marrow protein profile indicate an enhanced resorption and bone turnover following the loss of weight and reduction in BMD after surgery.

P 36 PLATELET LYSATE-BASED HYDROGELS INCORPORATED WITH MULTI-BIOACTIVE MESOPOROUS SILICA NANOPARTICLES FOR STEM CELL OSTEOGENIC DIFFERENTIATION

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BACKGROUND: Stem cells differentiation is usually performed in vitro, by exposing the stem cells to specific factors. Alternatively, one can use carriers containing such factors, which can be internalized by the cells. Bioactive silica mesoporous nanoparticles (MSN) containing calcium and phosphate ions on their surface and dexamethasone in their pores (MSNCaPDex) were synthesized. These three factors, once released inside adult stem cells, induce bone cell proliferation and differentiation, and stimulate the expression bone-related proteins. Besides being bioactive and biocompatible, methacryloyl platelet lysates (PLMA) hydrogels have mechanical properties that enhance cell adhesion and proliferation.

OBJECTIVES: By adding the MSNCaPDex to the PLMA structure, the differentiation of human bone marrow stem cells (hBM-MSCs) will be induced without the need of further osteogenic differentiation.

MATERIALS AND METHODS: Platelet-lysate-based hydrogels were constructed by mixing PLMA with hBM-MSCs and MSNCaPDex. After adding a photo initiator, the hydrogel reticulates under UV light, encapsulating the cells and nanoparticles. Cell studies are carried on for 21 days in basal medium and no other osteogenic supplementation. The controls consist on PLMA hydrogels in osteogenic medium (positive control) and basal medium (negative control).

RESULTS: Live/Dead assays performed after 1, 7 and 14 days showed that the cells remain viable and present an enlongated morphology. To study the hBM-MSCs differentiation, immunocytochemistry of

osteopontin, mineralization imaging and Alizarin Red S staining were performed after 21 days. While there is no differentiation in the basal medium, both PLMA hydrogels in osteogenic medium and PLMA+1%NPs hydrogels show production of osteopontin and signs of matrix mineralization given by the hydroxyapatite and calcium nodes appearance. Also, Alkaline Phosphatase (ALP) and Bone Morphogenetic Protein 2 (BMP-2) were quantified. In the presence of 1%NPs the values of ALP and BMP-2 are like those of osteogenic medium and higher than basal medium (Day 14).

CONCLUSIONS: Stem cell osteogenic differentiation is induced by the presence of MSNCaPDex with comparable values to the osteogenic medium. The encapsulation of MSNCaPDex in the PLMA hydrogels is enough to guide stem cell differentiation without any further osteogenic supplementation.

P 37 GUIDED BONE REGENERATION USING A TITANIUM DIOXIDE SCAFFOLD – A PRE-CLINICAL STUDY

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BACKGROUND: Alveolar bone resorption has been demonstrated in both vertical and horizontal following tooth loss. The reduced bone volume often poses a challenge for implant-retained fixed rehabilitation of masticatory function and aesthetics.

To increase the bone volume, various graft materials can be used for guided bone regeneration (GBR). Allografts or xenografts are frequently used as a scaffold to induce new bone formation. However, natural bone grafts can carry diseases from the graft to the host. There are also concerns regarding their reproducibility as the bone is harvested from different individuals and from different parts of that individual.

Synthetic bone graft substitutes may overcome these challenges, but also be mass-produced with tailored macroscopic and microscopic properties. A novel titanium dioxide scaffold has shown good mechanical properties, but also the ability to enable bone formation and angionesis in minipigs.

OBJECTIVES: The aim of this project is to evaluate the quantity of bone regeneration following treatment with a titanium dioxide scaffold or a bovine xenograft control (BioOss), in a non-acute lateral augmentation model.

MATERIALS AND METHODS: The hemimandibles of eight beagle dogs were randomly allocated to 4 or 12 weeks healing time. Three defect sites were created on the buccal side of the mandibles eight weeks prior to the GBR procedures. Three groups of materials were evaluated: The test group using a titanium dioxide scaffold block, a positive control using (BioOss) and negative control without any graft material. All three sites were covered with a collagen membrane (BioGide). The graft materials and membranes were placed on the buccal bone plate of the defects, to perform a lateral bone augmentation.

RESULTS: Following euthanization, all sites will be scanned using a micro-CT to create a 3D reconstruction. Volumetric analysis will also be done based on the CT reconstruction and 3D scanned casts from the impressions taken of the jaws before and after GBR. The models will be superimposed to assess the volume changes between different time points. Histological analysis will be performed by dividing each site in two halves for morphological analysis and immunohistochemistry. Antibodies

will be applied for the identification of osteopontin, matrix metallo-proteinases, angiogenesis and myeloperoxidase.

CONCLUSIONS: All the surgeries have been performed and healing has been uneventful. No complications have occurred and clinical evaluation of the 12 weeks healing group after 8 weeks of healing shows good soft tissue adaptation to the biomaterials.

P 38 THE CRITICAL EFFECTS OF MATRICES ON CULTURED CARCINOMA CELLS: HUMAN TUMOR-DERIVED MATRIX PROMOTES CELL INVASIVE PROPERTIES

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BACKGROUND: The interaction between cancer cells and the tumor microenvironment (TME), including the non-cellular extracellular matrix (ECM), plays an important role in cancer progression. Therefore, in order to develop cancer therapies, it is essential to understand the effects of ECM on cancer cells, since their response to these therapies is affected by the TME.

OBJECTIVES: We aimed to study the effects of different ECMs on head and neck squamous cell carcinoma (HNSCC) cell lines morphology, proliferation, migration and invasion.

MATERIALS AND METHODS: We cultured UT-SCC-24 (tongue), and UT-SCC-42 (larynx), including primary (A) and corresponding metastases (B) cell lines, on top and within 5 matrices: Matrigel, fibronectin, Myogel, fibrin and collagen. We observed the effects of these matrices on cell morphology using light and scanning electron microscopies. We measured their effects on cell proliferation using luminescent cell viability assay, and on cell migration and invasion using scratch wound healing assay.

RESULTS: We demonstrated that various ECM molecules significantly affected cells' behaviour. Carcinoma cells morphology was either flat, organoid or spindle depending on the matrix used. Fibrin enhanced, but collagen reduced the proliferation of all these cell lines. The effects on cell migration was cell line dependent. Interestingly, cells cultured within Myogel-collagen had the fastest invasion.

CONCLUSION: HNSCC cells behavior depended on different ECM matrices. The effects of ECMs were either constant on all cell lines, or cell line dependent. According to these results, we suggest to select a suitable matrix for each assay type, and for each carcinoma cell line, in order to receive the most reliable results.

P 39 IN-SILICO MODELLING AS AN EXPEDIENT TOOL FOR THE ASSESSMENT OF BIOREACTOR-INDUCED DYNAMIC CONDITIONS

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BACKGROUND: Recent novelty in tissue engineering includes the development of bioreactors, which enables scientists to reproduce a dynamic *in-vivo* condition in an *in-vitro* cell culture model.

This may improve the efficiency of cell expansion and promote targeted differentiation of mesenchymal stem cells more consistently. However, while different types of bioreactor systems have been introduced, the experimental evaluation of the dynamic condition that bioreactors generate is challenging. This is because each bioreactor system has distinctive and complex features, which hinder not only performing comparisons among previous studies but also elucidating the relation of causes and effects in the model. Therefore, it is an urgent agenda to establish methodology to assess bioreactor systems. Here, we suggest the utility of a *in-silico* mathematical model in the field of bioengineering.

OBJECTIVE: The most common function is the induction of fluid flow, mimicking *in-vivo* blood circulation. Generally, the magnitude of flow is described as a flow rate. To determine an ideal magnitude of flow, however, mechanical stress which cells receive from the flow is more attention-grabbing. The aim of current study is to computationally predict flow-induced shear stress applied to three-dimensional scaffolds in a bioreactor.

MATERIALS AND METHODS: Three dimensional microporous scaffolds of Poly(L-lactide-co-trimethylene carbonate) were prepared by a salt-leaching method, and the material evaluation was performed by micro‑computed tomography (micro-CT). A bioreactor, which can generate perfusion, was used as a model. The exact geometry of the bioreactor was reproduced in the *in-silico* simulation software, COMSOL Multiphysics®. The porosity, interconnectivity, density and permeability values from micro-CT analyses were applied to the scaffolds. Then, the flow dynamic state of the scaffold inside the bioreactor was simulated, and the bioreactor-induced fluid flow was calculated to predict shear stress and micro flow magnitude.

RESULTS: The *in-silico* simulation allowed to visualise the change of microfluidic flow in the bioreactor, showing that the scaffolds placed in it are exposed to differential shear stress dependant on their position. The amount of flow and shear stress gradually decreases as the flow goes into the scaffolds but is still continuous from the inlet to the outlet.

CONCLUSIONS: Computational simulation is an expedient tool to facilitate the understanding of complex dynamics generated by a bioreactor. This will result in elucidating the relationship between shear stress and cell behaviour. Moreover, the tool can be used to optimise the geometry of a bioreactor.

P 40 RETENTION OF BONE-SEEKING ALPHA PARTICLES IN INFLAMMATORY AND CALCIFYING TISSUE AROUND STENTS

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BACKGROUND: Arterial stents are implants used to support and maintain the structures of arteries. Plaque may form around stents following treatment of atherosclerosis as a result of chronic inflammation. This may lead to restenosis and heart failure. The use of drugs targeting inflammation and the calcification process seen in and around stents should be investigated as a possible treatment.

Radium, an alpha particle emitter, is of specific interest as it targets and binds to calcifying tissues. The alpha particles deliver high linear energy at a short range and induce double-strand breakage in the targeted cells.

OBJECTIVE: The objective of this study is to assess the targeting ability of radium to inflammatory and calcifying tissue in arteries. The study may have implication for future treatments that target the inflammatory tissue around stents.

MATERIALS AND METHODS: Explanted arteries or tissue samples from arteries were examined for symptoms of inflammation and plaque formation before testing the absorption and retention rate of radium-224 in the tissue. Arteries and tissue samples were exposed to the radionuclide by either dipping the tissue samples in a container with radium, or by flushing radium through the artery using a peristaltic pump or manually with a needle. Retention of radium was measured using a gamma counter.

RESULTS: Arteries and tissues displaying symptoms of inflammation and stenosis (such as blood clotting, plaque formation and calcification) displayed higher measurements of retained radium compared to arteries without symptoms of inflammation and stenosis. There were no significant difference in retention of radium between arteries of early stage stenosis and arteries with advanced calcified plaque.

CONCLUSIONS: The higher measurements of retained radium in arteries with stenosis suggest that radium is a prospective candidate for targeted treatment of restenosis in arteries. The retention seen in arteries with early stages of restenosis suggest that radium may be beneficial after stent placement, before significant plaque calcification.

P 41 CAN PORPYROMONAS GINGIVALIS CAUSE A PROGRESSION OF ALZHEIMER`s DISEASE AND CAN IT BE REVERSED BY LACTATE TREATMENT?

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BACKGROUND: One of the symptoms of Alzheimer's disease (AD) is inflammation in the brain. A cause of systemic inflammation is the bacterium causing periodontitis, *Porphyromonas gingivalis.* Recently, *P. gingivalis* has been observed in brain tissue of deceased AD patients. Moreover, it has been shown that brain inflammation caused by *P. gingivalis*-LPS infection can lead to learning and memory impairment in mice. Whether *P. gingivalis* can cause AD or lead to a progression of an already established disease is not known. Physical activity is recognized as anti-inflammatory and for increasing cognitive performance, which makes it suitable for treating AD. However, it can be challenging to get AD patients to do exercise. During physical exercise, lactate produced by muscles enters the brain and activate brain lactate receptors, HCAR1, belonging to a group of G-protein coupled receptors. Interestingly, lactate has been shown to protect against inflammatory damage in mice through activation of HCAR1, but the role of HCAR1 in regulating inflammation in the brain is unknown.

OBJECTIVES: Our aim is to find out whether *P. gingivalis* has a role in the progression of AD, and whether lactate treatment can be used as an alternative to physical exercise by protecting against inflammatory *P. gingivalis* reactions through activation of HCAR1.

MATERIALS AND METHODS: To find out whether *P. gingivalis* can increase inflammation in healthy or diseased brains, we use a model for periodontitis in mice, where live *P. gingivalis* or PBS control are applied repeatedly to the gingival margin of the maxillary molars of the AD mouse 5XFAD and wild type. In addition, to test whether signalling through HCAR1 is involved in the therapeutic action of lactate, we use a crossing of 5XFAD and a mouse with knockout of HCAR1. To mimic high lactate levels obtained during exercise, we administer lactate or PBS control to the mice once per day. After the treatment period, the cognitive abilities of the mice are tested in a Y-maze. In the end, we will analyze markers of inflammation in the brains by identifying activated microglia (Iba1) and astrocytes (GFAP) in addition to the cytokines TNF-alpha, IL-6, and IL-1beta using immunohistochemistry. We will also use PCR to detect and quantify bacterial DNA.

RESULTS AND CONCLUSIONS: Information we will obtain from these experiments will tell us whether lactate treatment working through activation of HCAR1 can counteract the inflammatory response in the brain and halt the cognitive decline caused by *P. gingivalis*. This knowledge can be used to develop new and improved therapeutics.