Research Day 2013

Faculty of Dentistry,
University of Oslo

29. May 2013
09.00 – 16.00

Store Auditorium

Institute of Clinical Dentistry
Geitmyrsveien 71
Research Day 2013
The Faculty of Dentistry, University of Oslo

09.00  Welcome

Session 1, moderator: post doctor Caspar Wohlfahrt

09.15  ECTODERMAL DYSPLASIA/OLIGODONTIA AND QUALITY OF LIFE. AN INTERDISCIPLINARY APPROACH
Solfrid Sørgjerd Saltnes, Dept of Oral Surgery and Oral Medicine, Institute of Clinical Dentistry

09.30  NEW ELECTRIC/MANUAL TOOTHBRUSH IN NURSING HOMES - A FOLLOW-UP STUDY
Kathrine Gare Fjeld, Dept of Cariology and Gerodontologi, Institute of Clinical Dentistry

09.45  BACTERIAL MICROFLORA IN ATHEROSCLEROTIC BIOPSIES FROM PATIENTS WITH AND WITHOUT PERIODONTITIS
Zahra Armingohar, Department of Oral Biology

10.00  PERIODONTAL PATHOGENS – A POSSIBLE ROLE IN CARDIOVASCULAR DISEASE?
Ingvild Kristin Midtervoll, Department of Oral Biology

10.15  THE HIPPO SIGNALING PATHWAY IS REQUIRED FOR SALIVARY GLAND DEVELOPMENT AND ITS DYSREGULATION IS ASSOCIATED WITH SJÖGREN-LIKE DISEASE
Tone Berge Enger, Dept of Oral Surgery and Oral Medicine, Institute of Clinical Dentistry

10.30  Coffee break

Session 2, moderator: post doctor Camilla Husvik

11.00  EFFECTS OF IN VIVO TRANSFECTION ANTIMIRs ON GENE EXPRESSION IN MURINE MOLAR TOOTH GERM
Natalie Sharim Skalleberg, Department of Oral Biology

11.15  SUBFRACTIONS OF ENAMEL MATRIX DERIVATIVE DIFFERENTIALLY INFLUENCE CYTOKINE SECRETION FROM HUMAN ORAL FIBROBLASTS
Oscar Villa, Department of Biomaterials, Institute of Clinical Dentistry

11.30  GRAIN BOUNDARY CORROSION IN TIO2 BONE GRAFT SUBSTITUTES
Benjamin Müller, Department of Biomaterials, Institute of Clinical Dentistry

11.45  ATYPICAL FEMORAL FRACTURE WITH ABNORMAL BONE CHARACTERIZED BY IMPAIRED MINERALIZATION
Maziar G. Shabestari, Department of Biomaterials, Institute of Clinical Dentistry

12.00 – 13:00 Lunch
Session 3, moderator: post doctor Ingvild J. Brusevold

13.00 EXPRESSION AND REGULATION OF THYMIC STROMAL LYMPHOPOIETIN ISOFORMS IN ORAL TISSUE
Louise Bjerkan, Department of Oral Biology

13.15 STREPTOCOCCAL CAPSULE SWITCH ACROSS THE SPECIES BORDER
Håkon V. Rukke, Department of Oral Biology

13.30 EVALUATION OF THE EFFECT OF FURANONE AND THIOPHENONE ON E.COLI MOTILITY
Ingun Lund Witsø, Department of Oral Biology

13.45 OPTIMIZATION OF STORAGE TEMPERATURE FOR HUMAN ORAL KERATIONCYTE CELL CULTURES
Rakibul Islam, Department of Oral Biology

14.00 THE PROGNOSTIC IMPACT OF SOX2 IN ORAL SQUAMOUS CELL CARCINOMAS
Cecilie Gjøvaag Attramadal, Department of Oral Biology

14.15 Coffee break

Session 4, moderator: post doctor Rasa Skudutye Rysstad

14.30 MAY ARTHROCENTESIS OF THE TEMPOROMANDIBULAR JOINT (TMJ) IN JUVENILE IDIOPATHIC ARTHRITIS PROVE TO BE AS GOOD AS INTRAARTICULAR GLUCOCORTICOID INJECTIONS?
Heming Olsen-Bergem, Dept of Oral Surgery and Oral Medicine, Institute of Clinical Dentistry

14.45 ANTERIOR TOOTH ALIGNMENT: A COMPARISON OF ORTHODONTIC RETENTION REGIMES FIVE YEARS POST-TREATMENT
Ragnar Bjering, Department of Orthodontics, Institute of Clinical Dentistry

15.00 PATIENTS OFFERED ORTHOGNATHIC SURGERY: WHY DO MANY REFRAIN FROM TREATMENT?
Niels Hågensli, Department of Orthodontics, Institute of Clinical Dentistry

15.15 HYPODONTIA: INTERDISCIPLINARY APPROACH TO TREATMENT OF 212 CHILDREN AND ADOLESCENTS
Christina Hvaring, Department of Orthodontics, Institute of Clinical Dentistry

15.30 Awards and closing session, professor Ulf Örtengren and professor Ivar Espelid
ECTODERMAL DYSPLASIA/OLIGODONTIA AND QUALITY OF LIFE. AN INTERDISCIPLINARY APPROACH

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Background: Oligodontia is characterized by the absence of six or more teeth (third molars excluded) and may be associated with Ectodermal dysplasias (EDs). EDs are a group of hereditary diseases and involve abnormal development of hair, nails, sweat glands and teeth. Decreased salivation has been documented which is significant for oral health. It is not uncommon that many teeth are missing, and present teeth are often pointed in shape. This may effect appearance, and not to mention oral function and also cause a physical and emotional problem for the patient. Ideal treatment of individuals with EDs requires experienced clinicians. It is important that the treatment planning is started early and that it is multidisciplinary.

Objectives: The aims of the present study are to assess how Oligodontia/ED affect oral health, treatment and quality of life.

Study group and methods: All patients with Oligodontia/ED, aged 5 years and older, registered at the TAKO-centre are included to participate in the study. Inclusion criteria are diagnosis of Oligodontia/ED, aged 5 years and older and ability to understand Norwegian. They have all been sent a questionnaire related to quality of life and dental treatment, see below.

Design: The study has a cross-sectional design. Data will be collected via questionnaires including demographics, psychical health, oral health, mental health, quality of life, coping and other self-reported factors. A clinical oral examination will be carried out and supplemented with orthopantomogram (OPG) and clinical photos. Completed treatment plans and treatment needs will be identified. Unstimulated and stimulated whole saliva will be collected using standard techniques. Anamnestic interviews and focus interviews will be carried out.
NEW ELECTRIC/MANUAL TOOTHBRUSH IN NURSING HOMES - A FOLLOW-UP STUDY

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Background: A two months RCT-study, with 180 nursing home residents, showed significant improvement in dental hygiene for both electric (ET) and manual (MT) toothbrushes.

Objective: To investigate if the improvement sustained in a longer perspective.

Methods: After the RCT-study, all participants were offered the choice between a new electric toothbrush and a new manual toothbrush. They were given the same 1450ppm NAF toothpaste. Examinations after one year were performed by the same dental team. Outcome was measured by the oral hygiene index (OHI-S).

Results: After one year 49 was dead and 30 dropped out. 101 participants were analyzed; mean age 86.5 (±8.3). 75% were women, >78% had three or more diagnoses and mean number of prescription drugs was 4.5 ±2.9. 47 (51.7%) had moderate to serious cognitive impairment. Mean number of remaining teeth was 18.8±6.0. 47 used ET, 54 MT. After RCT, OHI-S was further significantly improved, mean improvement was 0.21±0.51, no significant difference between ET and MT (p=0.44). At one year follow-up 39 (38.6%) had good oral hygiene, 59 (58.4%) moderate and 3 (3.0%) poor. (ET: good: 18 (38.3%), moderate: 29 (61.7%). MT: good: 21 (38.9%), moderate: 30 (55.6%), poor: 3 (5.6%).)

Conclusion: After one year, dental hygiene was significant improved to a level indicating high quality oral care. Appropriate and available dental aid equipment should therefore be mandatory in frail populations.
BACTERIAL MICROFLORA IN ATHEROSCLEROTIC BIOPSIES FROM PATIENTS WITH AND WITHOUT PERIODONTITIS

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An association between chronic infectious and inflammatory diseases, such as chronic periodontitis (CP) and atherosclerotic vascular diseases (ASVD) has been observed. The detection of periodontal pathogens in atherosclerotic lesions has suggested an involvement of these bacteria in atherogenesis.

Objective: The purpose of this study was to compare the bacterial microflora in specimens of atherosclerotic plaque removed from multiple sources such as abdominal aortic aneurysms and carotid or femoral arteries in patients with (w/) and without (wo/) CP using molecular microbiology.

Methods: DNA was extracted from atherosclerotic specimens from 30 patients w/CP and 10 wo/CP. Part of the 16S rRNA gene was amplified by universal primers and polymerase chain reaction (PCR). The PCR amplicons were cloned into Escherichia coli, sequenced, and identified by comparisons with known sequences in GenBank and the Human Oral Microbiome database. In addition 10 randomly selected vascular specimens from patients w/CP were subjected to scanning electron microscopy (SEM). Checkerboard DNA analysis was used to assess the presence of RCB in 10 randomly selected dental plaque samples from patients w/CP.

Results: A broader variety of bacteria, including common oral bacteria and numerous members of the family Enterobacteriaceae, were detected in patients w/CP compared to those wo/CP. Only one member of RCB, Porphyromonas gingivalis, was detected in one vascular specimen form a patient w/CP. The dental plaque samples from patients w/CP showed the presence of 1-3 members of the RCB group in 7 out of 10 samples. Bacteria were visualized in 80% of the vascular specimens by SEM.

Conclusions: Our results clearly showed a more diverse bacterial colonization in atherosclerotic lesions of patients w/CP compared to patients wo/CP. These findings indicate that a multitude of bacteria rather than single pathogens both from periodontitis and the gut may be involved as additional risk factors in the pathogenesis of ASVD.

Keywords: Chronic periodontitis, cardiovascular disease, bacteria, 16S rRNA
PERIODONTAL PATHOGENS – A POSSIBLE ROLE IN CARDIOVASCULAR DISEASE?

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Objective: The aim of this ongoing study is to investigate a possible role for periodontal pathogens in relation to cardiovascular disease (CVD) through detection of bacterial DNA in blood samples from participants of the Oslo II study.

Materials and methods: The study design is a blinded case-cohort design where the cohort is men from Oslo, born between 1926 and 1932. The test group consists of 225 individuals who have died from CVD (myocardial infarction, aortic aneurysms or stroke), and the control group are 225 healthy men randomly picked from the cohort. After DNA extraction from blood samples in both groups, PCR amplification of the 16S rRNA bacterial gene will be performed, and PCR products of 650bp fragment will be verified on a 1.5% agarose gel. Bacterial species will be detected by high throughput sequencing of the PCR products, and results from test and control group will be correlated. Furthermore, results from three diagnoses in the test group will also be correlated separately to the controls. Correction for known risk factors such as smoking, physical inactivity, high blood pressure, total serum cholesterol and diabetes will be performed.

Results: Preliminary results from PCR amplification of 260 blinded blood samples indicate bacterial DNA detection with the expected fragment size in 124 (48%) of the samples investigated so far.
THE HIPPO SIGNALING PATHWAY IS REQUIRED FOR SALIVARY GLAND DEVELOPMENT AND ITS DYSREGULATION IS ASSOCIATED WITH SJOGREN-LIKE DISEASE

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Sjogren’s syndrome (SS) is a complex autoimmune disease primarily affecting salivary and lacrimal glands leading to decreased secretion from exocrine glands. Although the prevailing dogma is that immune system pathology drives SS, increasing evidence points to structural defects to be involved in its etiology. Recently, the Hippo signaling pathway has emerged as one of the core pathways regulating cell proliferation and differentiation, in part via interaction with E-cadherin mediated junctions. Using ex vivo cultures of the embryonic mouse submandibular gland (SMG), we show that Hippo signaling is required for morphogenesis and organization of ductal structures. A Hippo effector, TAZ, interacts with E-cadherin and its junctional component, α-catenin, early in morphogenesis and this association increases along with cytodifferentiation. Inhibition of a Hippo pathway kinase, Lats2, abolishes ductal cell polarity and leads to precocious duct initiation. SMGs from NOD mice, a mouse model for SS, phenocopy the Lats2-inhibited SMGs. Importantly, labial specimens from human SS patients exhibit mislocalization of TAZ from junctional regions to the nucleus, coincident with accumulation of extracellular matrix components, fibronectin and Ctgf. Our studies show for the first time that Hippo signaling is required for SMG development and that defects in this pathway are associated with SS in humans.

References
EFFECTS OF IN VIVO TRANSFECTION ANTIMIRS ON GENE EXPRESSION IN MURINE MOLAR TOOTH GERM

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Objective: Micro RNAs are important during normal tooth development. The purpose is to study effects of different doses of anti-miR-214 and anti-miR-199 on gene expression and tooth phenotype in the first mandibular tooth germ.

Methods: CD-1 strain mice is used. On the first day of birth each of about 10 pups is injected with anti-miR-214 and anti-miR negative control (Ambion Austin TX) with doses of 50-200 pmol. Anti-miRs are chemically modified single stranded oligonucleotides designed to specifically bind to and inhibit endogenous miRNA. For the injections, the oligonucleotides are dissolved in in vivo jetPEI (Polyplus-transfection, Illkirch, France) which is an in vivo transfection reagent. After 24 hours the mice are killed and tooth germs was dissected out while the heads well immersed in RNA later solution (Ambion). miRNA enriched fractions are isolated from the tooth germ using mirPremier™ microRNA Isolation kit. The isolated miRNA is used to check the effect on the gene expression using microarrays (OneArray microarrays). cDNA was prepared using TaqMan® Gene Expression Assays and was analysed by Real time PCR assay-ViiA 7 Real Time PCR system. Statistical analysis was carried out by Spotfire and REST 2005 for microarray and RT-PCR data, respectively.

Results: The Anti-miR-214 injections resulted in inactivation of miR-214 in the tooth germ.

Conclusions and future work: The in vivo transfection with anti-miR-214 was succesful and showed that the higher dosage of anti-miR-214 had a stronger effect. Effects of transfection with other antimiRs (e.g. 199a 3p, 199a 5p) on gene expression and tooth phenotype. Effects of treatment with antimiRs on methylation status of genes subjected to epigenetic regulation will also be investigated (e.g. Ndn, Igf2, Dlk1).
SUBFRACTIONS OF ENAMEL MATRIX DERIVATIVE DIFFERENTIALLY INFLUENCE CYTOKINE SECRETION FROM HUMAN ORAL FIBROBLASTS

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Background: Enamel matrix proteins play a crucial role in the organization of the developing enamel and are also involved in the formation of acellular cementum during nascent tooth development. Enamel matrix derivative (EMD, Emdogain) is used to promote periodontal regeneration during the corrective phase of the treatment of periodontal defects. EMD contains a complex mixture of proteins derived from alternative splicing and controlled post-secretory processing, and the bioactive components of EMD remain obscure.

Objective: The aim of this study was to systematically analyze the effect of the different fractions of enamel matrix derivative on human primary periodontal ligament fibroblasts as measured on cytokine secretion.

Methods: EMD was subjected to size-exclusion chromatography, and a total of 13 5-ml fractions were collected. The fractions were characterized by SDS-PAGE. Human primary periodontal ligament (PDL) were treated with either EMD (10 µg ml-1) or the different fractions. Untreated cells were used as controls. Cell culture media was harvested after 1, 3, 7 and 14 days and the level of 25 cytokines was analyzed using the Luminex human cytokine/chemokine panel.

Results: The SDS-PAGE analyses showed that the most prominent bands were found between 25 kDa and 5 kDa. Fractions with proteins above 20kDa induced higher VEGF and IL-6 secretion, whereas lower molecular weight fractions enhanced the secretion of IL-8 and MCP-1 and reduced IL-4 and IL-7 release.

Conclusion: Our results indicate that higher molecular components in the EMD formulation might be responsible for VEGF and IL-6 secretion while lower molecular weights might be related to the expression of chemotactic factors, IL-4 and IL-7.

Key words: Emdogain; enamel matrix proteins; periodontal regeneration; wound healing; cytokines.
GRAIN BOUNDARY CORROSION IN TiO2 BONE GRAFT SUBSTITUTES

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Objective: The aim of this study was to investigate the sensitivity and the extent of grain boundary corrosion under physiological and non-physiological conditions for a ceramic scaffold.

Methods: 240 TiO2 scaffolds with known morphological and architectural properties were immersed in deionised water and phosphate buffered saline (PBS), respectively. Different pH conditions in the water solutions were obtained by adding 0.1 mol·l⁻¹ hydrochloric acid (HCl) and 1 mol·l⁻¹ sodium hydroxide (NaOH). PH values were adjusted to pH 3, pH 5 and pH 7. Time of immersion was set to 2, 4, 6, 8, 12 and 24 weeks. Each scaffold was submerged in 10 ml of the respective solution in individual polyethylene tubes. The study was conducted under static conditions of 37°C in an incubator. At the end of the respective time points the scaffolds were removed from the solutions and dried for 24h at 40°C. Subsequently scaffolds were tested under compressive load until failure. Following the mechanical tests scanning electron microscope (SEM) was used to assess the microstructural integrity of the scaffold, fracture surfaces and the grain boundary appearance. ANOVA was used to test the null hypothesis that there is no significant change in compressive strength among the different groups.

Results: Significant decrease in compressive strength (p<0.001) for all time points under strong acidic conditions (pH 3) was noticed. Medium acidic conditions (pH 5) and PBS exposure showed a gradually decrease in compressive strength over time, whereas neutral conditions showed no significant influence up to 12 weeks. SEM images of triple junctions in the ceramic boundary network support the assumption of grain boundary corrosion.

Conclusions: A remarkable drop in compressive strength was observed under pH 3 conditions, which already occurred within 2 weeks time. Long time exposure to medium acidic conditions and PBS showed similar results.

Keywords: Grain boundary corrosion, TiO2 scaffold, compressive strength
ATYPICAL FEMORAL FRACTURE WITH ABNORMAL BONE CHARACTERIZED BY IMPAIRED MINERALIZATION

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Objectives: Impaired bone material properties have been invoked as being responsible for the development of atypical femoral fractures (AFF) after long term bisphosphonate use. We therefore analyzed bone material properties in a bone biopsy obtained at the fracture site from an 88 year old female with AFF, who had been treated with alendronate for 8 years.

Methods: We used conventional histology, quantitative backscattered electron imaging (qBEI) and Raman spectroscopy (RS).

Results: Histology revealed numerous eroded surfaces, widened osteoid seams and osteocytic osteolysis. qBEI exhibited a scaffold of highly mineralized, porous bone matrix with numerous enlarged, osteocyte lacunae. Bone mineralization density distribution (BMDD) was shifted towards lower and more heterogenous mineralization compared to a normal reference: Mean calcium content (CaMean - 4.1% and CaPeak -1.8%), mineralization heterogeneity (CaWidth +29.3%), bone with reduced mineralization (CaLow +111%) and bone with increased mineralization (CaHigh -2%). RS data obtained at open osteons were compared with iliac crest biopsies from 35 healthy premenopausal, 16 treatment-naive osteoporotic women (PMC) and osteoporotic females (OP) treated with different bisphosphonates. The mineral/matrix ratio of AFF bone was similar to two alendronate and two risedronate groups, lower than PMC, and higher than either OP or OP-zoledronate groups. The proteoglycan content was higher in the AFF biopsy compared to all other groups. The mineral crystallinity of AFF bone was similar to both ALN groups, but lower compared to all other groups. Most significantly, however, we detected increased levels of pyrophosphate at osteoid/mineralized bone interfaces in AFF bone, a feature absent in other biopsies obtained from subjects after long term bisphosphonate treatment.

Conclusions: Bone from this case of AFF showed several abnormalities:
1) Altered arrangement of osteons; 2) Impaired mineralization; 3) Appreciable pyrophosphate accumulation, which might cause the impaired mineralization. Taken together, these changes may be responsible for the focally reduced bone strength in AFF.
EXPRESSON AND REGULATION OF THYMIC STROMAL LYMPHOPOIETIN ISOFORMS IN ORAL TISSUE

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Objective: Epithelial cells at mucosal surfaces are exposed to a variety of antigens, including pathogenic
and commensal bacteria, food antigens and allergens. The ability of the immune system of the oral
cavity to protect against infection while avoiding destructive inflammatory responses is essential for
maintaining oral health. Thymic stromal lymphopoietin (TSLP) is an epithelial-derived cytokine known to
activate dendritic cells and regulate immune responses in several tissues. The aim of this study was to
investigate the expression and regulation of the two TSLP transcript variants in oral epithelium and
cultured oral keratinocytes.

Methods: Biopsies were taken from the oral mucosa of healthy volunteers and from the gingiva of
persons with healthy gingiva or with periodontitis. The biopsies were processed for immuno-
histochemistry (IHC) and in situ hybridization (ISH). Oral mucosal biopsies were also used to establish
cultures of normal oral keratinocytes. The cultures were stimulated with various inflammatory
mediators and lysate of Porphyromonas gingivalis, and thereafter harvested for examination by real
time PCR and western blot.

Results: Protein and mRNA analyses showed that the short form TSLP (sTSLP) isoform is expressed in
mucosal and gingival epithelium. The levels were reduced in the periodontal pocket epithelium that
overlies the inflammatory infiltrate. In cultured oral keratinocytes exposed to poly(I:C), TNFα/IL-1β or
IFNγ, the expression of long form TSLP (lTSLP) transcript but not that of short form (sTSLP) transcript
was significantly increased.

Conclusions: lTSLP is the regulated form in oral keratinocytes. Due to its wide-spread expression in
gingival biopsies, sTSLP might have immunological or non-immunological roles in oral epithelia, e.g.
antibacterial activity, regulation of immune activity, modulation of barrier functions or epithelial
differentiation.
STREPTOCOCCAL CAPSULE SWITCH ACROSS THE SPECIES BORDER

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Objective: Capsule has long been known as a virulence hallmark in *Streptococcus pneumoniae*. Recent findings showing that the capsule locus is present in several strains of the predominant oral commensal *S. mitis*, a close relative of *S. pneumoniae*, prompted us to investigate whether genetic exchanges involving the capsule locus may lead to interspecies capsule switch, and if such an event will lead to altered pathogenicity.

Methods: A kanamycin and an erythromycin marker were inserted in the 5′- and 3′-ends of the capsule flanking regions of *S. pneumoniae* TIGR4. DNA from this mutant was used to transform *S. mitis* type strain. Production of the TIGR4 capsule by *S. mitis* was confirmed using the Neufeld capsule test. RAW macrophages were used to investigate phagocytosis of the *S. mitis* TIGR4 capsule expressing mutant, a capsule deletion mutant and the wild type. To study the *in vivo* effects of capsule and capsule switch, mouse models of early lung infection and septicemia were used.

Results: This is the first demonstration that capsule switch in streptococci may occur across the species border, between species with high differences in pathogenic potential. Our results show that acquisition and production of the *S. pneumoniae* TIGR4 capsule protected *S. mitis* against phagocytosis by macrophages *in vitro*, and against macrophage-mediated clearance in a mouse model of early lung infection. Interestingly, the TIGR4 capsule offered higher protection against phagocytosis than the native capsule. No significant differences were, however, observed in the septicemia model. Blood clearance rates in the first 30 min were, indeed, significantly higher for *S. mitis* than for *S. pneumoniae*, even by increasing the *S. mitis* inoculum 10 times above the *S. pneumoniae* inoculum.

Conclusion: Elucidating the consequences of virulence gene exchange is relevant from a clinical perspective. It is also of fundamental interest for understanding the remarkable ability of oral streptococci to colonize and survive as commensals in the human host, in contrast with *S. pneumoniae* that are occasional colonizers with a high pathogenic potential. Our results indicate that replacement of the native capsule by the *S. pneumoniae* capsule enhances the *S. mitis* ability to resist phagocytosis. Interestingly, the increased protection conferred by the *S. pneumoniae* capsule was not sufficient for *S. mitis* to achieve the clearance resistance levels of *S. pneumoniae*.
EVALUATION OF THE EFFECT OF FURANONE AND THIOPHENONE ON *E.coli* MOTILITY

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Objective: The aim of this study was to compare the effect of thiophenone TF101 and furanone F202 to function as quorum sensing inhibitors by evaluating their ability to interfere with motility in *Escherichia coli* O103:H2.

Introduction: Bacterial biofilms have become an increasing problem both in the medical treatment of infections as well as in industry. When bacteria grow in this sessile community, they often develop resistance to antimicrobial substances, disinfectants and human immune defense mechanisms. Biofilm formation requires expression of flagella involved in bacteria motility. Several studies have shown that bacterial communication is important in expressing virulence factors like flagella and in the establishment of a biofilm, suggesting that this could be a new target in the fight against bacterial biofilm infections. Previous studies have shown that furanone and thiophenone are able to interfere with biofilm formation conceivably by disrupting bacterial communication. Since motility is strongly associated to biofilm formation we wanted to study the effect of TF101 and F202 on motility in *E.coli*.

Methods: Motility-plate assay and scanning electron microscopy (SEM) were used to study effect of TF101 and F202 on motility and expression of flagella at non-toxic concentrations (10 μM). Real time PCR was used to study the effect of TF101 on expression of *flhD*, encoding one subunit in the master regulator for flagella transcription.

Results: TF101 reduced motility in motility-plate assay, and SEM images indicated that expression of flagella was impaired upon treatment with TF101. This was not observed in F202 at the concentrations tested. Real-time PCR showed that TF101 reduced expression of *flhD* in cells in exponential phase.

Conclusion: Our results show that TF101 reduce motility in *E.coli* O103:H2 by inhibit flagella expression, confirmed by phenotypic and genotypic testing. F202 had no effect on *E.coli* motility at the concentration tested.
OPTIMIZATION OF STORAGE TEMPERATURE FOR HUMAN ORAL KERATINOCYTE CELL CULTURES

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Objective: Human oral keratinocyte (HOK) cell cultured sheets have recently proved hugely successful in the treatment of stem cell deficiency of the ocular surface, and may hold great potential also for other disorders. Storage technology is mandatory to enable transportation of (HOK) cells to clinics all over the world, hence increasing the availability of this promising treatment. We, therefore, assessed the effect of different storage temperatures on the viability, morphology and phenotype of cultured HOK cells.

Methods: Cultured HOK cells were stored in HEPES and sodium bicarbonate buffered MEM at nine temperatures (4°C, 8°C, 12°C, 16°C, 20°C, 24°C, 28°C, 32°C and 37°C) for seven days. Viability was assessed by a microplate fluorometer and the metabolic status (pH, glucose, lactate, pCO2, pO2) was evaluated using Radiometer ABL 700, Morphology was analyzed by scanning electron microscopy.

Results:
Viability was significantly reduced in all storage groups except 12°C compared with unstored control cells. Cell survival was highest after storage at 12°C (84% ± 33% compared with unstored cells; P<0.001 compared to 4°, 8°, 20°, 24°, 28°, 32° and 37°C; P = 0.585 in between 12°C and 16°C; P=0.532 compared to unstored) and 16°C (68%±18.42% compared with unstored cells; P<0.039 compared to 4°, 8°, 20°, 24°, 28°, 32° and 37°C; and P=0.003 compared to unstored). Presence of Glucose and lactate concentration in the media showed significant (P<0.001 in both cases) correlation to the viability, where lactate showed negative and glucose showed positive correlation with correlation coefficient value of -0.488 and 0.574 respectively.

Conclusions: Out of nine temperatures tested between 4°C and 37°C, storage at 12°C was the only temperature that maintained the morphology and viability. Thus, the ability to conserve HOK cells is critically dependent on storage temperature.
THE PROGNOSTIC IMPACT OF SOX2 IN ORAL SQUAMOUS CELL CARCINOMAS

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Objective: The transcription factor SOX2 plays central roles in embryo development and neurogenesis, cell differentiation, and in cancer development. However, so far the significance of SOX2 in development and progression of oral squamous cell carcinomas and response to primary treatment is unknown.

Material and methods: Tumour-tissue from 65 patients with primary, small oral squamous cell carcinoma (OSCC), T1/T2, N0, M0, was collected from ENT department, Rikshospitalet, Oslo. After diagnosis, the patients were primarily treated with surgery; and more than 50% later on with radiotherapy. Clinical information and follow up were obtained from the medical records. H&E staining was performed for histological reclassification and grading. Immunohistochemistry was performed for SOX2 proteins. The findings were correlated with clinicopathological features, treatment and follow-up information.

Results: Most of the carcinomas stained positively for SOX2. For cases treated with surgery and postoperative radiotherapy in combination, we found that patients with intense SOX 2 staining responded significantly better to treatment than cases with less intense staining (P-value 0.001). For cases treated with surgery alone, SOX2 expression was not associated with treatment response. SOX2 was observed in cell nuclei of morphologically normal epithelium, endothelium and salivary glands as well as in OSCC cells. We registered a trend towards better prognosis in cases where nuclei in tumour cells were more intensely stained than the normal epithelium.

Conclusions: SOX2 was expressed in the majority of OSCCs. We observed a trend towards better prognosis for patients with higher staining intensity in the tumours when compared to the intensity in the normal epithelium. These patients had a significantly better response on postoperative radiotherapy and better survival than patients with less intense staining.
MAY ARTHROCENTESIS OF THE TEMPOROMANDIBULAR JOINT (TMJ) IN JUVENILE IDIOPATHIC ARTHRITIS PROVE TO BE AS GOOD AS INTRAARTICULAR GLUCOCORTICOID INJECTIONS?

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Aim: The temporomandibular joint is not uncommonly affected (15-70%) in children with Juvenile idiopathic arthritis (JIA), often asymptomatic, but pain and limited function is not uncommon. The purpose of this study was to evaluate and compare the effect of intra-articular temporomandibular joint arthrocentesis to a combination of arthrocentesis and glucocorticoid-injections (triamcinolon) in patients with juvenile idiopathic arthritis (JIA).

Material and method: The study is a single-blinded prospective trial of 21 JIA patients (40 TMJ), 15 girls and 6 boys, age 6-18. Inclusion criteria was TMJ arthritis (mean duration JIA 5.1 years, TMJ pain/limitations 2.1 years), pain and limited movement of the mandible. Joints were randomly selected for either arthrocentesis or arthrocentesis/glucocorticoid injection. Follow-up at 3 and 8 months included measurements of pain intensity, pain localization, joint sounds, mandibular function and complications.

Results: Pain incisal opening (PIO), and maximum incisal opening (MIO), increased on first and second follow-up (PIO 19.0, 35.9 and 39.9mm)(MIO 26.7, 38.3 and 40.4mm). Lateral excursion increased from baseline 4.8 mm to 8 mm on the first and 8.8 mm on second follow-up. Measurements (VAS) of pain and function decreased from baseline 49 mm (pain) and 41 mm (function) throughout the first (18 mm and 19 mm, respectively) and second follow-up (8mm and 4 mm).

Conclusion: There is a great benefit of treatment with TMJ arthrocentesis in JIA, and surprisingly, adding glucocorticoid gave no additional effects. These improvements seem to last for months after one treatment only, and do not seem to be affected by the fluctuation of the disease in general.
ANTERIOR TOOTH ALIGNMENT: A COMPARISON OF ORTHODONTIC RETENTION REGIMES FIVE YEARS POST-TREATMENT

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Aim: To assess the influence of different retention strategies on anterior tooth alignment five years post-treatment, comparing three retention protocols for both the maxilla and the mandible.

Materials and Methods: 171 patients (75 male, 96 female) were analyzed using the peer assessment rating (PAR) index at four stages: pretreatment (T0), post-treatment (T1), 3 years (T3) and 5 years post-treatment (T5). Three retention protocols were identified in each jaw. In the maxilla: 1) removable retainer, 2) removable retainer + fixed retainer till T3, 3) removable retainer + fixed retainer till T5. In the mandible: 1) no retainer, 2) fixed 3-3 retainer till T3, 3) fixed 3-3 retainer till T5. Upper and lower anterior component PAR scores (ACS) were analyzed between the groups at each stage.

Results: ACS for upper anterior segment showed no significant differences between the retention protocols at any time point. No significant differences between the retention protocols in the lower anterior segment were seen at T1, however at T3 and T5 the no-retention group demonstrated significantly greater scores than the fixed retainer group. At T5, significant differences were seen between the group that had the retainer removed at T3 and the group that kept the retainer at T5.

Conclusion: Stability of the maxillary anterior alignment 5 years post-treatment did not appear to be influenced by choice of retention protocol. Mandibular anterior alignment was significantly better for the group using fixed retainer, compared to the group without retention and the group where the retainer was removed 3 years post-treatment.
PATIENTS OFFERED ORTHOGNATHIC SURGERY: WHY DO MANY REFRAIN FROM TREATMENT?

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Aim: After consultation about half of the referred subjects who were offered treatment refrain from having orthognathic surgery. The aim was to examine factors associated with patients’ decision to decline surgery.

Material/Methods: Of 470 consecutive patients referred to the University of Oslo from 2007 to 2009, a sample of 160 subjects who had not undergone surgery was identified and contacted. 236 operated patients from the same period served as a comparison group. Morphology was assessed from cephalograms and photographs, and the individuals’ opinions were recorded using questionnaires.

Results: Dentofacial morphology represented normative treatment need and was generally similar except for a higher rate of severe negative overjet in the operated sample ($P < 0.001$). About 90 per cent of the subjects were satisfied with the information they had received about surgery. The decision about treatment was characterised as difficult by 76.2 and 37.8 per cent by the un-operated and operated subjects, respectively. The most prevalent reasons for declining surgery were risks for side effects, the burden of care, and a general reluctance to undergo surgery. Many un-operated subjects were dissatisfied with their masticatory function, facial appearance and position of their teeth. Eighteen per cent responded that they probably would elect surgery in the future.

Conclusion: Informed consent to orthognathic surgery represents a challenge both to the patient and the professional. The findings imply that patients’ motives and fears should be explored during consultation and that the information provided should be adapted to the potential risks and benefits related to the actual treatment.
HYPODONTIA: INTERDISCIPLINARY APPROACH TO TREATMENT OF 212 CHILDREN AND ADOLESCENTS

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Objectives: To report clinical characteristics and treatment plans in a series of hypodontia patients referred to an interdisciplinary team, and to estimate the prognosis of retained primary mandibular molars.

Materials and Methods: The sample consisted of 212 patients with hypodontia, 115 girls and 97 boys, referred to the University of Oslo between 1998 and 2010. Information regarding severity of hypodontia, location of the missing teeth and treatment plans was obtained from patient files and radiographs. Panoramic radiographs were used to estimate the prognosis of retained primary mandibular molars lacking successors in terms of infraocclusion, root resorption and restorations in 111 patients.

Results: The mean age at time of referral was 13.2 years (range 6.3 – 29.8). The mean number of missing teeth per subject was 7.4 (range 1 – 23). Severe hypodontia, with more than six teeth missing, was found in 61.3% of the patients. Mandibular second premolars were most frequently missing, followed by maxillary second premolars and maxillary laterals. For children and adolescents, the most frequently recommended treatments were orthodontic appliance therapy (82%), leaving persisting primary teeth in situ (59%), composite restoration (31%), extraction of primary teeth followed by monitoring (28%), temporary Maryland bridge (14%) and autotransplantation (10%). Dental implants were planned in adulthood for 78% of the patients. Persisting primary molars showed clinically significant infraocclusion in 43.6% of the patients. Root resorption and restorations were of limited clinical importance.

Conclusions: Almost 80% of the patients needed interventions from three or more disciplines, emphasizing that hypodontia requires a complex and resource-intensive treatment. Orthodontic therapy and dental implants were the most commonly prescribed treatments. Retained primary molars were estimated to have a moderate prognosis, with infraocclusion as the most limiting factor.