

**Research Day 2014**

**Faculty of Dentistry, University of Oslo**

**23. May 2014**

**09.00 – 15.00**

**Department of Oral Biology**

**Domus Odontologica**

**Auditorium A1.1001**



## **Dear Participants,**

Welcome to "Research Day". The aim of the Research Day is to give each PhD candidate the opportunity to present their research project to a larger audience than their own research group and prepare for presentation at research congresses abroad. Furthermore, it is a good opportunity to get an overview of the Faculty's ongoing research projects.

The audience will be fellow PhD candidates as well as the Faculty's scientific staff and teachers. We hope they will support the PhD candidates and attend as many of the presentations as possible.

The abstracts of the research projects that will be presented, can be found in this program in order of their presentation time.

The organization committee of the Research Day has appointed professor Hilde Galtung, IOB, Faculty of Dentistry, University of Oslo and professor Daniela Elena Costea, Department of Clinical Medicine, Faculty of Medicine and Dentistry, University of Bergen as scientific evaluation committee. At the end of the day, two awards will be presented in accordance with the evaluation from the scientific committee.

We hope you will enjoy the day together with us and take part in exciting presentations and fruitful scientific discussions.

Dean of Research, Faculty of Dentistry  
Professor Ståle Petter Lyngstadaas

## Research Day 2014

### Programme

- 08:30 Registration
- 09.00 Welcome, by the faculty's Dean of research, professor Ståle Petter Lyngstadaas  
*Session 1, moderator: post doctor Carl Hjortsjø*
- 09.15 CLINICAL EFFECTIVENESS OF TWO ANTIMICROBIAL IRRIGANTS IN ENDODONTICALLY TREATED TEETH  
*Homan Zandi, Institute of Clinical Dentistry*
- 09.30 ANALYSIS OF THE CSP AND XIP SIGNALING PATHWAYS IN *S. MUTANS*  
*Rabia Khan, Department of Oral Biology*
- 09.45 *STREPTOCOCCUS MITIS* TRANSCRIPTOME CHANGES IN RESPONSE TO A NOVEL PHEROMONE  
*Roger Junges, Department of Oral Biology*
- 10.00 Coffe break  
*Session 2, moderator: post doctor Rasa Skudutyte-Rysstad*
- 10.15 DENTIST-SPECIFIC EFFECTS ON THE LONGEVITY OF DENTAL RESTORATIONS  
*Andreas Dobloug, Institute of Clinical Dentistry*
- 10.30 DENTISTS' EDUCATION AND THEIR USE OF BEHAVIOURAL MANAGEMENT TECHNIQUES  
*Kjetil Strøm, Institute of Clinical Dentistry*
- 10.45 SOUR SWEETS AND ACIDIC BEVERAGES AS RISK INDICATORS FOR EROSION  
*Jenny Bogstad Søvik, Institute of Clinical Dentistry*
- 11.00 Coffee break  
*Session 3, moderator: post doctor Hanna Tiainen*
- 11.15 BIOMOLECULAR COATING OF NICKEL-TITANIUM SHAPE MEMORY METALS  
*Simon Longela, Institute of Clinical Dentistry*

- 11.30      GRAIN BOUNDARY CORROSION IN TiO<sub>2</sub> BONE GRAFT SUBSTITUTES  
*Benjamin Müller, Institute of Clinical Dentistry*
- 11.45      THE ORAL COMMENSAL BACTERIAL SPECIES *STREPTOCOCCUS MITIS* SHOWS A TH17-  
DOMINATED MEMORY T CELL SIGNATURE THAT IS SIMILAR TO AND CROSS-REACTIVE  
WITH *STREPTOCOCCUS PNEUMONIAE*  
*Stian André Engen, Department of Oral Biology*
- 12.00      CHARACTERIZATION OF BIOLOGICAL EFFECTS OF ENAMEL PROTEIN AMELOBLASTIN  
*Øystein Stakkestad, Institute of Clinical Dentistry*
- 12.15      Lunch  
  
*Session 4, moderator: post doctor Linda Z. Arvidsson*
- 13.15      EGF FAMILY AND CISPLATIN RESISTANCE IN ORAL SQUAMOUS CELL CARCINOMA  
*Jian Gao, Department of Oral Biology*
- 13.30      TUMOR BUDDING IN ORAL CANCER  
*Cecilie Gjøvaag Attramadal, Department of Oral Biology*
- 13.45      COMPARATIVE ANALYSIS OF PULP SPACE IN CONTRA-LATERAL PREMOLARS  
*Gaute Floer Johnsen, Institute of Clinical Dentistry*
- 14.00      Coffee break
- 14.30      AWARDS AND CLOSING SESSION  
Professor Daniela Elena Costea and professor Hilde Galtung

## Clinical Effectiveness of Two Antimicrobial Irrigants in Endodontically Treated Teeth

Homan Zandi, DDS, Renata C. V. Rodrigues, DDS, Anne-Karin Kristoffersen, PhD, Dag Ørstavik, DDS, PhD, José F. Siqueira Jr, DDS, PhD, Isabela Rôças, DDS, PhD, Morten Enersen, DDS, PhD

### Abstract

**Background:** Endodontic treatment of root-filled teeth with post-treatment disease is especially challenging. The outcome of endodontic treatment is significantly influenced by the presence of bacteria in the root canals at the time of filling. Sodium hypochlorite (NaOCl) is widely used as a root canal irrigant in different concentrations because of its pronounced antimicrobial activity and the ability of dissolving necrotic tissue and organic matter. Chlorhexidine gluconate (CHX) is an effective substitute for NaOCl due to its broad-spectrum antimicrobial efficacy against bacteria and its high dentin substantivity. It is less toxic against vital tissues than NaOCl but lacks tissue dissolving property. No culture-independent molecular study has compared the antimicrobial effectiveness of 1% NaOCl and 2% CHX in root-filled teeth with apical periodontitis (AP). **Objectives:** The aim of the present study was to compare the antibacterial properties of 1% NaOCl and 2% CHX in endodontically treated teeth with AP. **Methods:** Sixty-eight root-filled teeth with AP were randomly distributed in two irrigant groups. Bacterial samples were taken at baseline (S1), after chemomechanical preparation using either 1% NaOCl or 2% CHX as irrigant (S2), and after intracanal medication with calcium hydroxide at the second visit (S3). The bacterial samples were analyzed by 16S rRNA gene-based quantitative polymerase chain reaction (qPCR) for total bacteria, *Enterococcus faecalis* and *Streptococcus* spp.. **Results:** Preliminary results indicated bacterial DNA in all the samples at S1. *E. faecalis* was seldom found, but streptococci were frequent. The amount of bacterial DNA significantly declined following treatment in both groups (S2 and S3) ( $p < 0.001$ ). There was no significant difference between NaOCl and CHX groups in reducing total bacterial and streptococcal counts ( $p > 0.05$ ). **Conclusion:** Findings indicate that endodontic retreatment was equally effective in reducing the amount of bacteria in both irrigation groups.

### Keywords:

Endodontics. Root canal irrigants.

# Analysis of the CSP and XIP signaling pathways in *S. mutans*

Khan R\*, Rukke HV, Høvik H, Chen T, Petersen FC

**Introduction:** In *Streptococcus mutans*, an oral colonizer associated with dental caries, the competence system is unique among streptococci, as it can be triggered by both CSP and XIP pheromones. CSP is sensed by a histidine kinase of the ComDE two-component system, whereas XIP is sensed inside the cells following internalization by the Opp permease. CSP has been shown to activate expression of the XIP encoding gene (*comS*), but the mechanism(s) is unknown. **Objectives:** Our aim was to gain an overall insight into how the CSP and XIP pheromone regulatory pathways are temporally linked. We mapped the global changes in gene expression at early and late phases of the CSP response and investigated the effect of *comS* deletion on the *S. mutans* transcriptional profile. **Methods:** *S. mutans* was grown in TSB or CDM in the presence or absence of CSP. pVA838 was used to monitor transformation levels. Labeled RNA was directly hybridized to a customized array. **Results:** In the wild type, transformation efficiency was increased by more than 300 fold in the presence of CSP, whereas in the *comS* mutant no transformants were observed. The early phase of the CSP response was characterized by an increase in gene expression at 4 loci, including bacteriocin-related genes. In the late phase, the up-regulated regions were extended to include a total of 27 loci, including *comS* and genes required for DNA uptake and recombination. Increased XIP production in the presence of CSP was verified using a reporter assay. **Conclusion:** In conclusion, our study provided temporal information on the gene expression profile of *S. mutans* in response to CSP and showed that the late response to CSP depends on the expression of *comS*. The findings support the model that in *S. mutans* the XIP pheromone has a central role in induction of competence development by CSP.

## ***Streptococcus mitis* transcriptome changes in response to a novel pheromone**

Junges R<sup>1</sup>, Rukke HV<sup>2</sup>, Aamdal HA<sup>1</sup>, Chen T<sup>3</sup>, Petersen FC<sup>1</sup>

<sup>1</sup>*Department of Oral Biology, University of Oslo, Oslo, Norway*

<sup>2</sup>*NIOM, Oslo, Norway*

<sup>3</sup>*The Forsyth Institute, Boston, USA*

### **Abstract**

**Objective:** The aims of the study were to investigate the possible presence of a small hydrophobic peptide (SHP) pheromone system in *Streptococcus mitis* type strain NCTC 12261, and to investigate transcriptome changes in response to the novel pheromone. **Methods:** Genome analysis of the sequenced *S. mitis* strain NCTC 12261 was conducted in two-steps: (1) identification of streptococcal Rgg homologues, and (2) search for open reading frame (ORF) encoding oligopeptides in the rgg flanking regions. Real-time PCR was used to investigate whether the identified oligopeptide DIIIVGG fulfilled the criterion for autoinducing activity that characterizes pheromone systems. Transcriptome analysis using RNA sequencing was performed for a comprehensive assessment of changes in gene expression in response to the synthetic pheromone. **Results:** Genome analysis revealed a non-annotated gene coding a possible novel pheromone (N-DIIIVGG), which was upstream of an Rgg transcriptional regulator. RT-PCR results showed that gene expression of the pheromone was increased by seven-fold in samples where the synthetic pheromone was added in comparison to the ones without it, indicating an autoinducing effect. Transcriptome analysis revealed that twelve genes were up-regulated at least two-fold by the new pheromone. The highest induction was observed for gene 0092 (55.9-fold) which codes a hypothetical protein with unknown function. This gene forms probably an operon, together with the 0093 gene, which codes for an ABC transporter ATP-binding protein (possibly multidrug efflux), and the pheromone gene. This last one was located in the intergenic sequence between 0093 and the rgg gene (ID0094). Transcription of the Rgg regulator was found to occur in the opposite direction of the pheromone operon. **Conclusion:** We identified a novel pheromone system in *S. mitis* that induces expression of more than ten genes. Next efforts will be directed to elucidate which specific functions this pheromone is taking part in and how it affects *S. mitis* group behavior.

**Keywords:** pheromone, bacterial signaling, genetics, streptococcus.

Presenter: Andreas Dobloug

Title: Dentist-specific effects on the longevity of dental restorations

Abstract

Objective: To estimate dentist-specific variation in the longevity of restorations in the first permanent molars for children aged 6-18 years in Norway.

Method: The dataset encompassed all children born in 1993 who received one or more restorations in their first permanent molars. This encompassed 72 573 restorations for 64 728 children who were treated by 2 473 public dental officers. The data were analysed using two multilevel models – mixed-effects logistic regression and mixed-effects linear regression.

Result: The dentist-specific variation was low. The intraclass correlation coefficient varied from 0.03 to 0.08 in different specifications.

Conclusion: Public dental officers in Norway show a high degree of homogeneity with regard to longevity of restorations. Further improvements in the longevity of restorations can most likely be achieved by preventing secondary caries and by improving the physical properties of materials.



## **Dentists' education and their use of behavioural management techniques**

Kjetil Strøm\*, Anne Rønneberg, Anne Skaare, Ivar Espelid, Tiril Willumsen

Dept. of Paediatric Dentistry and Behavioural Science, Institute of Clinical Dentistry, Faculty of Dentistry, University of Oslo

**Objective:** To explore the relationship between dentists' education in management of dental anxiety (DA) and their choice of behavioural management techniques (BMT).

**Methods:** An anonymous questionnaire was sent electronically to 611 dentists in the Public Dental Health Service in Norway questioning the use of BMT when treating children and adolescents with DA. Statistical evaluation was performed using cross-tabulation with chi-square and logistic regression analyses.

**Results:** The response rate was 65%. The majority of the 391 respondents graduated from Norwegian dental schools (74 %, n= 288), the remaining abroad (26 %, n= 101). Fifty-three percent (n= 208) had followed postgraduate courses in management of DA patients. The most frequently used BMT was tell-show-do (87%, n= 340), followed by relaxation (35%, n= 132), distraction (25%, n= 94), cognitive behaviour therapy (22%, n= 84) and conscious sedation (18%, n= 69). Factors associated with a high risk of using few techniques when treating DA patients was: graduating outside Norway (OR 2.8, 95 % CI= 1.6-4.8, p<0.001) and having not attended postgraduate courses in DA (OR 2.0, 95% CI= 1.3-3.2, p= 0.002).

**Conclusion:** Dentists' education was associated with amount and frequency of BMT used when treating patients with DA. Increased focus on education in behavioural science in the undergraduate curriculum and in postgraduate courses may help to reduce DA in children and adolescents.

**Key words:** Dental anxiety, Behavioural management techniques, Dentist-patient relationship, Dental education, Dentist attitude.

## **SOUR SWEETS AND ACIDIC BEVERAGES AS RISK INDICATORS FOR EROSION**

**J.B. SØVIK, R. SKUDUTYTE-RYSSTAD, A.B. TVEIT, L. SANDVIK & A. MULIC**

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**Objective:** The study aimed to investigate the association between dental erosion and potential background, behavioural and dietary risk indicators, and to assess whether there is a dose-response relationship between the level of acidic beverage consumption and dental erosion.

**Methods:** Of 846 adolescents (aged 16-18 years) scheduled for dental recall examinations, 795 (94%) accepted to participate and were examined for dental erosive wear on index surfaces using the Visual Erosion Dental Examination system (VEDE). Those with three or more surfaces with dental erosion were defined as affected individuals. All participants completed a self-administered questionnaire regarding their background (gender and age), tooth brushing frequency and dietary habits (the amount and frequency of acidic food and beverage consumption, as well as the chosen method and manner of consuming acidic drinks). For assessment of acidic drink intake, the Total intake Erosive Drinks (TED) index was computed, based on the aggregated daily consumption of four acidic drinks (fruit juice, soft drink, diet soft drink, sports drink). The index values were trichotomized into low (0-0.24 l/day), moderate (0.25-0.74 l/day) and high consumption (0.75-5 l/day). The association between presence of erosive lesions and the possible risk indicators were assessed by chi-square test and logistic regression analysis.

**Results:** Of all participants examined, 37% were affected individuals. Multivariate logistic regression analysis revealed a significant association between dental erosion and high consumption of sour sweets and sports drinks as well as moderate/ high total consumption of acidic drinks. A linear dose-response trend was found between the daily consumption of acidic drinks and dental erosion.

**Conclusions:** Dietary habits, in particular high consumption of sour sweets and acidic drinks, appear to be significant risk indicators for dental erosion. A linear dose-response relationship between the daily consumption of acidic drinks and dental erosion was detected.

## *Biomolecular coating of Nickel-Titanium shape memory metals*

**SIMON LONGELA\***, Håvard J Haugen\*, S. P. Lyngstadaas\*, Janne Reseland \*, Hana Tiainen\*

*\*Department of Biomaterials, Faculty of Dentistry, University of Oslo, Norway*

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### **Background:**

A simple yet effective surface coating procedure for implants is highly demanded. Here we are presenting a method which combines chemical surface modification with a bioactive coating of synthetic peptides on shape memory nickel-titanium stents to improve re-endothelialization ability and simultaneously reduce rate of stenosis and restenosis or no inflammatory potential.

### **Methods:**

To bring biomolecules on a surface of a metal requires the identification and understanding of the protein behaviour. A mathematical model algorithm has been developed to mimic the biomolecules folding and unfolding mechanism.

Various methods for immobilizing biomolecules on implant surface have been investigated among them: dipping into peptide buffer solution, covalent anchoring by silane agents, covalent binding of alkanethiols, trisyl-chloride activation, spacer assisted adsorption, and oligonucleotide mediated adsorption.

The specific aims:

- Identify the pH- region where the binding is optimal
- Finding the optimal current density and time point for adding biomolecules
- Find concentration of biomolecule on the surfaces with given processing parameters
- Investigate release kinetics of the biomolecules
- In vitro experiment in order to verify which concentration could have highest clinical effect

### **Summary:**

This study focuses on improving the concept of peptide coating on Nickel-titanium stents to promote effective arterial repair by computer simulations and in vitro experimentations.

This experimental study develops an easy and practical coating for Nickel-titanium shape memory and superelastic implants with specific synthetic peptides by using an advanced anchor system to promote re-endothelialization of artery wall tissue.

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**KEYWORDS:** Shape Memory Alloys, Nickel-Titanium, Surface characterization

## GRAIN BOUNDARY CORROSION IN TiO<sub>2</sub> BONE GRAFT SUBSTITUTES

**B. MÜLLER, J. WENGENROTH, H. TIAINEN and H. HAUGEN**

*Department of Biomaterials, Institute for Clinical Dentistry, University of Oslo, Norway*

**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:** TiO<sub>2</sub> 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<sup>-1</sup> hydrochloric acid (HCl) and 1 mol·l<sup>-1</sup> 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 microscopy (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 (pH3) 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, TiO<sub>2</sub> scaffold, compressive strength

**The oral commensal bacterial species *Streptococcus mitis* shows a Th17-dominated memory T cell signature that is similar to and cross-reactive with *Streptococcus pneumoniae***

Stian A. Engen<sup>1</sup>, Håkon V. Rukke<sup>1</sup>, David Jarrossay<sup>2</sup>, Inger J. Blix<sup>1,3</sup>, Federica Sallusto<sup>2</sup>, Fernanda Petersen<sup>1</sup>, Karl Schenck<sup>1</sup>

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**Abstract**

The commensal bacterial species *Streptococcus mitis* is a predominant colonizer of the human oral cavity and pharynx. Carriage of and infection with the closely related pathogenic *Streptococcus pneumoniae* induce specific Th17 memory responses, but the presence and type of Th memory cells showing reactivity towards commensal streptococci with low pathogenic potential is largely uncharacterized. In this study, the natural Th cell memory immune response to *S. mitis* was studied. Blood from healthy donors was used to establish Th memory cell libraries. Cultures enriched in Th subset-specific cells based on chemokine receptor expression were polyclonally expanded before they were characterized for streptococcal reactivity. The Th memory cell signature in the Th1 (CXCR3+CCR6-), Th1\* (CXCR3+CCR6+), Th2, Th17 and Th22 subsets obtained for *S. mitis* was compared to that of *S. pneumoniae*. Among the subsets, both species revealed mixed and similar signatures with predominant Th17 and Th1\* responses, the frequencies of reactive cells being consistently higher for *S. pneumoniae*. Reactive Th17 cells were cloned to screen for interspecies reactivity and cross-reactivity was observed. The similar and cross-reactive T cell memory signature of *S. mitis* and *S. pneumoniae* suggests that carriage of commensal and pathogenic bacterial species can influence each other reciprocally.

## Characterization of biological effects of enamel protein Ameloblastin

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Øystein Stakkestad<sup>1</sup>, Jiri Vondrasek<sup>2</sup>, Jan Oxhold Gordeladze<sup>1</sup>, Ståle Petter Lyngstadås<sup>1</sup>, Janne Elin Reseland<sup>1</sup>.

<sup>1</sup>Department of biomaterials, Institute of Clinical Odontology, University of Oslo, Norway, <sup>2</sup>Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Flemingovo nam, Prague, Czech Republic.

**Background:** Regulation of biomineralization is a highly regulated process dependent on many factors and diverse processes. Ameloblastin (AMBN) has been thought of as an enamel specific protein but was previously shown expressed in mesenchymal stem cells and is implicated in differentiation of mesenchymal stem cells and promoting biomineralization. *In vivo* Ambn is cleaved into many small fragments that may function differently depending on cleavage pattern.

**Objective:** The aim of this study was to compare biological effects of AMBN variants and peptides on mesenchymal stem cells growth and differentiation.

### Materials and methods:

Human Mesenchymal stem cells (hMSC) (Cat. # PT-2501; Lonza) were maintained in mesenchymal stem cell medium (Lonza) from passage 5. Medium were changed every 3<sup>rd</sup> day and passaged at 90 % confluency to either 12 well plates, 24 well plates (Milliplex analysis and mRNA isolation) or 48 well plates (Proliferation, Thymidine incorporation). Secretion profiles of the media over the cells were analysed with the Luminex-200 system using the HCYTOMAG-60K and the HBNMAG-51K kits (MILLIPORE corporation, Billerica, MA, USA) with antibodies against IL-6, IL-8, VEGF, Leptin, SOST respectively. Rawdata were analysed with the Luminex xPONENT® version 3.1.871 or MILLIPLEX™ Analyst version 5.1 software. Real time PCR measurements of Osteocalcin and Collagen I was normalized against  $\beta$ -Actin and Glyceraldehydphosphate (GADPH) using the  $\Delta\Delta$ CT method.

**Results:** Proliferation was enhanced with with full-length AMBN compared to untreated cells. Expression of Leptin, IL-6 and SOST was different between N terminus and C terminus and Full-length AMBN at day 14. IL-8 expression at day 1 for N terminus was enhanced 2.5-fold but fell to a 1.5 fold plateau during the experiment while full-length AMBN was able to enhance IL-8 expression 4-fold at day 14. The early (2 and 24 hours) gene expression of Osteocalcin and Collagen I was most enhanced with short peptide segments based on the exon 5 and the isoform 1 compared to full-length and C and N terminal AMBN variants.

**Conclusion:** The various deletion of Ambn had different effects on differentiation, however full-length AMBN variant was required for proliferation of hMSC.

Keywords: Biomineralization, mesenchymal stem cells, proliferation, differentiation and bone

## EGF FAMILY AND CISPLATIN RESISTANCE IN ORAL SQUAMOUS CELL CARCINOMA

*J Gao<sup>1</sup>, CH. Ulekeiv<sup>1</sup> and TS. Halstensen<sup>1</sup>*

*<sup>1</sup>Department of Oral Biology, Faculty of Dentistry, UIO*

**Objective:** Cisplatin is one of the most important chemotherapeutic drugs used in clinical treatment for oral squamous cell carcinoma (OSCC). However, some OSCC developed cisplatin resistance within 6 months, which is a major cause of therapy failure. Cancer produce high level of epidermal growth factor (EGF) family members show decreased response to cisplatin. In this study, we investigated the EGF family members in OSCC, and identified key proteins that may be related to cisplatin resistance.

**Methods:** 11 OSCC cell lines were exposed to different dosages of cisplatin. Half maximal inhibitory concentration (IC<sub>50</sub>), cell survival ratio after exogenous ligands and inhibitors were quantified by the XTT assay. The mRNA and protein expression of EGFR, ERBB2 were analysed using qRT-PCR and western blotting, respectively. Furthermore, the expression of relative proteins in the downstream of ERBB2 signaling pathway was detected by western blotting.

**Results:** The expression of EGFR had a negative correlation with cisplatin resistance both in mRNA and protein level, and no pronounced influence on cisplatin induced cytotoxicity could be detected after treatment with both EGFR ligands and inhibitor. In contrast, the expression of ERBB2 showed a positive correlation with cisplatin resistance. Moreover, ERBB2, MEK, p38 and JNK inhibitors had synergistic effect with cisplatin. The signaling activity in the MAPK pathways decreased after ERBB2 inhibition.

**Conclusion:** High expression level of ERBB2 shows a poor response to cisplatin in OSCC cell lines, but no such pattern has been observed in the expression of EGFR in contrast to breast cancer. ERBB2 inhibition can reverse cisplatin resistance by altering MAPK pathways, suggesting ERBB2 probably plays a key role in cisplatin resistance in OSCC.

## Forskningsdagen 23.mai 2014

Cecilie Gjøvaag Attramadal<sup>1</sup>, Sheeba Kumar<sup>1</sup>, Hari Prasad Dhakal<sup>2</sup>, Morten E. Boysen<sup>3</sup>, Jahn M. Nesland<sup>2</sup>, Magne Bryne<sup>1</sup>. (1) *Institute of Oral Biology, Faculty of Dentistry, UiO.* (2) *Dep. of Pathology, Oslo University Hospital, The Norwegian Radium Hospital, Faculty of medicine, UiO.* (3) *ENT department, Oslo University Hospital, Rikshospitalet, Faculty of medicine, UiO.*

### Abstract

**Title:** Tumor budding in oral cancer

**Objective:** Small oral squamous cell carcinomas (OSCC) are characterized by a highly variable prognosis, and new prognostic factors are needed to give the patients optimal treatment. Budding is defined as a small group of tumor cells, buds, with 1-5 cells at the tumor/stromal border, and is accepted as a prognostic factor in colo-rectal cancer. The purpose of this study was to evaluate if tumor budding is a prognostic factor in OSCC.

**Materials and methods:** Tumor-tissue from 58 patients with primary, small oral squamous cell carcinoma (OSCC), T1/T2, N0, M0, was collected from the ENT department, Rikshospitalet, Oslo. After diagnosis, the patients were primarily treated with surgery; and 62% with postoperative radiotherapy. Clinical- and follow-up information was obtained from the medical records. Immunohistochemistry was performed using anti-Pan Keratin antibodies, and the observation of tumor budding was scored in the most invasive part of the tumor, registering number of buds at 200x magnification. The findings were correlated with treatment and follow-up information.

**Results:** All carcinomas stained positively for Pan-Keratin. The tumors were split into two groups, <5 buds or ≥5 buds. The number of buds were correlated with follow-up (time to recurrent disease) and treatment. There was a significantly higher number of recurrences among patients with ≥ 5 buds,  $p = 0.043$ . Budding was not significantly associated with treatment.

**Conclusion:** Tumor budding in the tumor front was detected in most of the OSCCs, and high number of buds was associated with recurrent disease, indicating a clinical value.



## XXXXXXXX COMPARATIVE ANALYSIS OF PULP SPACE IN CONTRA-LATERAL PREMOLARS

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Friday, May 23, 2014: 08:30 a.m. - 16:00 p.m.

Location: Store Auditorium, Institute of Oral Biologi (Domus Odontologica)

Presentation Type: Oral Session

**G. F. Johnsen<sup>1,2</sup>, J. Wengenroth<sup>1</sup>, D. Ørstavik<sup>2</sup>, H. J. Haugen<sup>1</sup>, S. P. Lyngstadaas<sup>1</sup>**

<sup>1</sup>Department of Biomaterials, Institute for Clinical Dentistry, University of Oslo, Norway

<sup>2</sup>Department of Biomaterials, Institute for Clinical Dentistry, University of Oslo, Norway

This study has been evaluated and deemed not to necessitate disclosure by The National Committee for Medical and Health Research Ethics (NEM) of Norway (reference number: 2012/2092b – Remit Assessment).

**Objective:** The aim of the first study is to determine the degree of similarity *in silico* of the pulpal space in extracted contra-lateral human premolars. These are to be used in a second study in order to establish if roots need to be considered identical before they are used in endodontic leakage tests. The null-hypothesis ( $H_0$ ) is that contra-lateral premolars are not identical.

**Methods:** 41 pairs of contralateral mandibular or maxillary premolars were selected from 36 volunteer patients referred for extraction from the Section of Orthodontics, University of Oslo. Immediately following extraction, micro-CT scans using the Skyscan 1172  $\mu$ CT system (SkyScan, Kontich, Belgium) were conducted. Analyses with both commercial and customized software were performed in order to determine to what degree the contralateral premolars were identical. Parameters evaluated for determination of similarity included volume ( $v$ ), surface ( $s$ ), surface-over-volume ( $sov$ ), degree of anisotropy ( $doa$ ), curvature and torsion ( $ct$ ), and three-dimensional metrology ( $3dm$ ), and visual inspection of 3D-renderings. The teeth were then stored in an ethanol humidifier (70%) at constant temperature in the Department of Biomaterials' cold room awaiting root canal preparation, obturation, micro-CT scanning, and leakage testing.

**Results:** Contralateral premolars, in general, show a high degree of similarity for the parameters  $v$ ,  $s$ ,  $sov$ , and  $doa$ . For instance, the mean and standard deviation (mean (SD)) values for  $s$  (14vs24, 15vs25, 34vs44, 35vs45) were 0.9674 (0.0349), 0.9424 (7.6946e-3), 0.9257 (0.0661), and 0.9546 (0.0575), respectively. Preliminary findings for  $ct$  and  $3dm$  show the same trends in similarity. However, there were several examples of contra-lateral premolars exhibiting extremely different values among the chosen parameters.

**Conclusions:** Micro-CT scanning for analysis of pulp space anatomy proves to be a non-invasive method for determining similarity among extracted contralateral premolars. The null-hypothesis is tentatively rejected.

**Keywords:** Micro-CT, pulp space anatomy, comparative anatomy