

## 1. Mechanism of Palatal Epithelial Seam Disappearance with Overexpression of Smad2

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**Objectives:** Cleft palate is a common birth defect. In analyses of non-syndromic cleft palate, a linkage to TGF- $\beta$ 3 has been shown. Signalling of TGF- $\beta$  is mediated in the cell through Smad2. Ultimately TGF- $\beta$  signalling leads to the disappearance of the epithelial midline seam and the confluence of the mesenchyme. TGF- $\beta$ 3 null mice have a cleft secondary palate, a phenotype that has been rescued by targeted overexpression of Smad2 in the MEE. The goal of this research was to understand the mechanism of palatal fusion in the rescue mice compared to wild-type.

**Methods:** The heads of embryos at age (E14.5) of three different mice models (rescue, Smad2 overexpression and wild-type) were embedded in paraffin after genotyping and fixation. Serial 7 micron sections were studied for detection of apoptosis and epithelial mesenchymal transition using immunohistochemistry. Images were captured with confocal laser microscope. Activation of Smad2 was studied with phospho-Smad2 antibody, and the level of Smad2 in each embryo normalized with immunoblotting.

**Results:** TGF- $\beta$ 3 null mice developed a secondary palatal cleft while the TGF- $\beta$ 3 null mice that had also inherited the K14-Smad2 gene had fusion of the secondary palate. The effect of the K14-Smad2 expression was analyzed in the medial edge epithelium of the rescue mice; the MEE had a much higher ratio of cells with cleaved caspase, a marker of apoptosis, than in the control fused palates. The increase in apoptosis was correlated with increased p-Smad2 in the same cells. Increased p-Smad2 in the control mice with normal palatal fusion was not associated with high levels of apoptotic MEE.

**Conclusions:** Smad2 overexpression might rescue the cleft in the secondary palate of mice by increasing apoptosis of epithelial cells in the middle seam. Thus the mechanism of rescue is not identical to the events that normally occur during palatal fusion.

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## 2. **The Role of Smad2 Overexpression and the Progression of Periodontitis**

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**Objectives:** Periodontitis is a chronic inflammatory disease, characterized by destruction of the periodontal attachment apparatus including the alveolar bone. Previous studies have indicated the involvement of TGF- $\beta$  signalling in periodontitis progressions. TGF- $\beta$  signalling is responsible for a variety of cellular processes such as proliferation, differentiation and apoptosis. The Smad2 transcription factor lies at the heart of TGF- $\beta$  intracellular mediators. Previous authors have reported the effect of Smad2 overexpression on multiple mouse tissues (Ito *et al.* 2001), but none of those studies have reported the role of Smad2 overexpression on the progression of periodontal disease. We hypothesized that Smad2 overexpression will cause attachment-loss periodontitis.

**Methods:** We used K14-Smad2 transgenic mice to investigate the effect of Smad2 overexpression in the periodontium and compared it to wild-type mice at 1, 3, 6, 9 and 12 months. Detection of attachment loss was done by using photographs of hemi-sectioned mouse maxillae stained by Van Gieson and ponceau stains for detecting bone and cementum. MicroCT analysis was performed to quantify the level of alveolar bone. The apoptosis index and the mitotic activity of junctional epithelium were measured by TUNEL analysis and immunofluorescence for PCNA respectively.

**Results:** Our preliminary results show that K14-Smad2 mice had more bone loss (47.75%) with exposure of the roots when compared with the wild-type mice that had 19.5% of their roots exposed. There was a significant difference when comparing the apoptotic index of the junctional epithelium of K14-Smad2 mice with the wild-type mice at 35.99% and 2.98%, respectively. An opposite outcome was observed comparing the mitotic activity; wild-type mice had a significantly higher activity (20.79%) when compared to the K14-Smad2 mice (1.31%).

**Conclusions:** Smad2 overexpression causes periodontal attachment loss with increased apoptosis and reduced mitotic activity of the junctional epithelium.

**Acknowledgements:** This research was conducted as part of Dr. Mazen Alotaibi's PhD research at the University of British Columbia, Vancouver, Canada.

### 3. **Temporary Anchorage Devices in Orthodontics**

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**Objectives:** To study orthodontic management of an extracted ankylosed tooth and closure of the remaining space with Temporary Anchorage Devices (TADs) as absolute anchorage instead of conventional orthodontic mechanics.

**Methods:** A 13-year-old healthy male with good oral hygiene, class II subdivision left malocclusion, ankylosed 36, mesioangular 37, developing 8's and overerupted 26. Tooth 36 was extracted and tooth 37 was first uprighted and then protracted to substitute 37 for 36 and also 38 for 37. Tooth 26 was intruded to the upper occlusal plane for ideal levelling of the occlusal plane. Forsus devices were used on the left side to correct the midline discrepancy and class II subdivision.

**Results:** A poor quality tooth (ankylosed 36) was removed and replaced by a healthy tooth. The patient will benefit from the elimination of the cost of restoration and long-term restorative care. The lower-left third molar was saved and present in functional occlusion. The orthodontic treatment time was longer.

**Conclusions:** When considering the ideal treatment for a patient, one should consider the best possible and latest technology and techniques. This case report demonstrates that a TAD can serve as an absolute anchorage to protract molars for closing space without having any negative effect on the occlusion of contralateral side or opposing arch.

#### 4. Chemotactic Response of Macrophages to Surface Roughness

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**Objectives:** Recent *in vivo* studies (Chehroudi 2010) showed a higher macrophage density and faster mineralization on rough sandblasted and acid-etched (SLA) surfaces compared to smooth (Po) substrata. It is known that macrophages can polarize into different phenotypes, which either promote inflammation (classical) or wound healing (alternative) through the release of specific cytokines. The goal of this study was to examine *in vitro* if surface roughness affects the secretion of cytokines and chemokines by macrophages.

**Methods:** Murine macrophages (RAW267.4) were cultured for 1 and 5 days on Po or rough SLA substrata. As controls, RAW264.7 cells were primed with either LPS and IFN- $\gamma$  or IL-4 into the inflammatory or alternative phenotype. Culture supernatants were collected to screen the secretion profile of the RAW264.7 cells with an antibody array (Proteom Profiler<sup>TM</sup>, R&D Systems) for 40 different cyto- and chemokines. Chemokines, whose secretions were shown to be influenced by surface roughness, were quantified by ELISAs.

**Results:** The general secretion profile of RAW264.7 cells on Po and SLA surfaces was very similar to the IL-4 primed alternative activated cells with a high secretion of the macrophage-attractant chemokines MIP-1 $\alpha$  and MCP-1. In contrast, the LPS/ IFN- $\gamma$  primed cells showed a high secretion of MIP-1 $\alpha$  and MCP-1, but also other chemokines such as MIP-1 $\beta$ , MIP-2, RANTES and IP-10. On SLA, the secretion of MIP-1 $\alpha$  and MCP-1 was elevated compared to the Po sample, which was particularly pronounced after 5 days. ELISAs quantitatively confirmed these qualitative results from the antibody arrays.

**Conclusions:** The up regulation of the macrophage-attractant chemokines MIP-1 $\alpha$  and MCP-1 on SLA agrees with the observations from Chehroudi *et al.* who observed *in vivo* a remarkably higher macrophage density on SLA substrates compared to Po, which coincided with early bone formation. Therefore, an elevated secretion of MIP-1 $\alpha$  and MCP-1 could be beneficial to osseointegration.

**Acknowledgements:** Supported by the Canadian Institutes of Health Research and the UBC Faculty of Dentistry Research Fund.

## 5. **Risk Factors for Oral Cancer Development: Lessons from History**

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**Objectives:** The establishment of a risk predictive model can have important public health implications. Health professionals have used reliable risk prediction tools for breast and lung but oral cancer lacks such a model. The goal of this study was to review all of the 1995-1998 mucosal biopsies from the BC provincial oral biopsy service and to link patient and lesion information to outcome data.

**Methods:** Reports of the mucosal biopsies from 1995-1996 were manually selected and scanned, and electronic data queried from the Pathology database for 1997-1998. This was then incorporated with information collected from the clinicians' requisition forms for patient demographics and lesion characteristics. Personal health numbers were used to identify outcomes via linkage to the Pathology database and the BC Cancer Registry from 1995 to 2010.

**Results:** 1,605 out of 5,881 mucosal biopsies contained clinically white lesions including hyperplasia with no dysplasia (728, 45%), lichen planus (399, 25%), dysplasia (290, 18%), cancer (106, 7%), and candidiasis (82, 5%). We identified 15 (~1%) cases which had progressed to cancer and surprisingly, five of them had no dysplasia. With a location at the gingiva/buccal mucosa, they were anatomically at low-risk, and two of them had an obvious history of oral lichen planus. There was significant site predilection among lichen planus, hyperplasia, and dysplasia (Tongue/FOM: 19%, 12%, 43%, respectively,  $P < 0.0001$ ). The data support that the presence of dysplasia on the tongue/floor of the mouth remains the best risk indicator (5%) and gingival lichen planus (0.15%) has a slightly elevated cancer risk.

**Conclusions:** This is the first step to build a database for this unique, 30-year population-based material. With the analysis of this enriched archive material (patient and lesion information and archive paraffin tissue), we will be able to better understand the nature and history of the disease.

**Acknowledgements:** Supported by the Canadian Cancer Society Research Institute (CCSRI-20336); the Canadian Institutes for Health Research, the Michael Smith Foundation for Health Research, and a UBC Faculty of Dentistry Undergraduate Student Summer Research Award.

## 6. **Impact of Nodal Status on Survival of Oral Cancer Patients**

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**Objectives:** Oral squamous cell carcinoma (OSCC) is notorious for having a poor prognosis, mainly due to regional failure. The goal of this study was to examine the nodal status and its impact on survival, and to identify nodal-metastasis-predictive markers at the time of diagnosis.

**Methods:** A retrospective cross-sectional study was performed on patients with primary OSCC from 1995-2008 who received surgery with intent-to-cure, with  $\geq 12$  months follow-up. Data collected included: smoking habit, anatomical site, nodal status at/after surgery, and outcomes in regional failure and survival. A total of 283 identified patients were categorized into 4 groups: A. Patients who were LN- at surgery or during follow-up (N=183); B. Patients who were LN- at surgery but LN+ during follow-up (N=56); C. Patients who received concurrent neck dissection and were LN+ at the time of surgery (N=39); and D. Patients who received concurrent neck dissection and were LN- at the time of surgery (N=5).

**Results:** There is no difference among groups in demographics, smoking habits, and anatomical sites. Nodal status at or after surgery is significantly associated with survival (Groups A to D, 75%, 43%, 46%, and 100%, respectively), time-to-survival (Groups A&D, 83.5 $\pm$ 44.5 months vs. Groups B&C, 53.4 $\pm$ 46.5 months,  $P < 0.0001$ ) and 5-year survival (Groups A&D, 84%, vs. Groups B&C, 47%,  $P < 0.0001$ ). There is no difference in time-to-death and survival rate between Groups B and C. Strikingly, among 239 patients in Groups A and B, despite the pre-surgery screening, 23% developed regional failure, with 80% at the first 18-month follow-up.

**Conclusions:** Patients' survival is highly associated with the nodal status. An effort to identify effective markers in clinical, pathological and molecular levels to predict nodal disease pre-surgery is ongoing. This will benefit a quarter of early-stage patients from prophylactic neck dissection, and ultimately lead to a better survival.

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7. **Klearway™ Appliances for Class II Division I Pediatric Orthodontic Patients**

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**Objectives:** The Klearway™ appliance was designed to open the airway by gradual advancement of the mandible. It has been used successfully for adult patients with obstructive sleep apnea and/or snoring. The functional effects of Klearway™ on mandibular growth in pediatric patients with Angle Class II, Division 1 malocclusions have not been investigated. This preliminary study assessed how Klearway™ could be used as a functional appliance.

**Methods:** Patients were selected to participate from the undergraduate orthodontic program at UBC. The criteria for each patient were: Angle Class II Division 1 with a retruded mandible, upright and well-aligned mandibular incisors, a deep overbite ( $\geq 20\%$ ) and a deep overjet ( $\geq 4$  mm). The baseline data included study models, cephalometric and panoramic X-rays, together with intra-oral and extra-oral photos. In addition, a sleep questionnaire was administered at baseline. A customized Klearway™ was fabricated for each patient and a portable sleep monitor (Watch-Pat) was used on the insertion night. Patients were advised to wear Klearway™ at night only. The patients were treated by monitoring and/or adjusting Klearway™ on a monthly basis through to the Phase II comprehensive treatment stage. Follow-up records were obtained to verify the craniofacial changes and sleep quality.

**Results:** No patients discontinued therapy due to appliance discomfort. Eighteen patients (8 girls and 10 boys) completed Phase I treatment. The average baseline age was 12 years 0 months. The Angle's classification transitioned to Class I in 16 patients and Class III in 2 patients. The overjet was significantly decreased from  $7.0 \pm 2.4$  mm to  $3.0 \pm 2.3$  mm ( $p < 0.001$ ). The overbite was decreased significantly from  $59.4 \pm 23.6\%$  to  $28.1 \pm 19.4\%$  ( $p < 0.001$ ). There were no significant findings in questionnaire scores and sleep analysis.

**Conclusions:** Klearway™ is a suitable functional appliance for pediatric patients who exhibit Class II Division 1 malocclusions and retruded mandibles.

**8. Understanding the Gaps: Resources and Marginalized HIV/AIDS Communities in Vancouver**

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**Introduction:** A large proportion of HIV-seropositive members from Aboriginal populations and Latino illegal immigrants/refugees are failing to utilize HIV resource services, remaining at risk for further health struggles.

**Objectives:** To engage these HIV-seropositive groups in a dialogue about their needs and barriers in utilizing HIV resource services.

**Methods:** Peer-led focus-group discussions and individual interviews occurred within each group following an interview guide. Through constant comparison, field notes and voice-recorded information were analyzed thematically and inductively for codes, themes and categories. A literature review identified relevant information to support and refute such findings.

**Results:** Two focus groups and one interview of 70 minutes in average length occurred during Spring 2011, and of a total of 17 participants enrolled; most reported being MSM (men who have sex with men). The thematic analysis revealed the existence of similar concerns on issues of empowerment (as people attempt to learn about themselves and the disease to get better control of their health); resources (as allocation of information helps them to get educated about the health care system); and support (either from their families, each other, friends or the community in which they are immersed). However hand, while the Latino group voiced the necessity of having language-specific psychological support to better understand their health condition, the Aboriginal group claimed to be financially deprived.

**Conclusions:** HIV resource organizations can tailor their services to address the needs of hard-to-reach individuals who are HIV seropositive. These organizations can help marginalized subpopulations to maintain their health and curb the spread of the disease.

**Acknowledgements:** This study was funded by a UBC Faculty of Dentistry Undergraduate Student Summer Research Award and the Vancouver Coastal Health Authority via the STOP (Seek and Treat for Optimal Prevention of HIV/AIDS) Project.



9. **A Review of the Oral Manifestations of HIV**

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**Background:** A Rapid HIV Screening Test initiative in dental clinics was introduced in four different sites in Vancouver over the Summer of 2011. This initiative has a large educational component aimed at developing a resource booklet about the oral manifestations of HIV infection and AIDS for clinical and non-clinical staff and patients.

**Objectives:** To compile the most relevant information on the oral manifestations of HIV infection and AIDS.

**Methods:** A thorough review of the literature was undertaken via PubMed, Google Scholar and Ovid, limited to publications available on-line in English and involving humans. Different sets of key words were used including 'HIV' and 'Oral manifestations', 'HIV' and 'Dentistry'; and so on. For each oral manifestation found, five categories of information were set up: nature of the manifestation, clinical appearance, differential diagnosis, diagnosis and treatment.

**Results:** A total of 3444 publication titles were gathered and 3033 excluded. The 411 titles accepted lead to the inclusion of 104 abstracts. Upon gathering the full text of the included abstracts, 63 on-line publications were included as part of this literature review. More than 20 different oral manifestations of HIV infection and AIDS were found and categorized as viral, fungal, bacterial, neoplastic, and others (non-specific agents) in origin.

**Conclusions:** A wide spectrum of oral manifestations can affect HIV-positive patients, sometimes as the first sign of HIV infection; therefore, early detection is crucial in optimal patient care. The information compiled is now being used in developing a resource booklet for clinical and non-clinical staff and patients.

**Acknowledgements:** The Rapid HIV Screening Test has been funded by the Vancouver Coastal Health Authority via the STOP (Seek and Treat for Optimal Prevention of HIV/AIDS) Project. Support for this project was provided by a UBC Faculty of Dentistry Undergraduate Student Summer Research Award.

## 10. Characterization of Gingival and Dermal Substitutes for Wound Healing

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**Objectives:** Gingival wounds heal without scars while skin wounds heal with scarring. Gingival fibroblasts and the extracellular matrix they produce are believed to contribute to scarless healing in the gingiva. The long-term goal of this project is to transfer fibroblasts from gingiva to skin wounds and explore whether they can prevent scarring in the skin. The aim of this project was to create gingival and dermal substitutes *in vitro* by culturing fibroblasts from both tissues on a polyglactin 910 mesh (Vicryl; Ethicon), and analyze their gene expression compared to cells grown on tissue-culture plastic. We hypothesize that both dermal and gingival fibroblasts can be used to generate tissue substitutes but that these cells display different gene expression profiles during generation of the tissue substitutes.

**Methods:** Fibroblasts from one line of dermal and gingival cells were cultured in 8-well dishes either on a polyglactin-910 (Vicryl) mesh or on plastic (control) at a density of 50,000 cell/cm<sup>2</sup>. Cells were cultured in DMEM containing 10% FBS and 50 micrograms/mL ascorbic acid to promote extracellular matrix deposition. After 7, 14 and 28 days, cultures were analyzed by scanning electron microscopy (SEM), Western Blotting and RT-PCR.

**Results:** SEM analysis showed that both dermal and gingival fibroblasts attached and grew on the Vicryl mesh and that fibroblasts oriented themselves along individual fibres of the mesh. Gene expression differed between dermal and gingival fibroblasts regardless of whether they were cultured on the Vicryl mesh or cell-culture plastic. Gingival and dermal fibroblasts cultured on the mesh showed differences in gene expression, especially at the later time points of cell culture.

**Conclusions:** Morphologically, gingival and dermal fibroblasts behave similarly on the Vicryl mesh, while at the molecular level, there are differences in gene expression.

**Acknowledgements:** Supported by the Canadian Institutes of Health Research and two UBC Faculty of Dentistry Undergraduate Student Summer Research Awards.

## 11. **Dental Students' Perceptions toward Simulation Teaching and Learning**

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**Objectives:** This study was a first attempt at eliciting DMD students' perspectives toward pre-clinical learning, in particular clinical simulation, using a validated survey. We compared the student survey results with educational literature, aiming to enhance our pre-clinical education.

**Methods:** In March 2008, all third and fourth year DMD students at UBC were invited to participate in the Simulation Teaching and Learning (STL) survey. Among the invited students, 34 students from the Class of 2008 and 28 students from the Class of 2009 completed the survey. At the time of data collection, students from third year had nearly finished all of their pre-clinical training. The fourth years had nearly completed a year of intensive clinical training. Embedded in the STL survey were students' perceptions toward various learning modes, interactions with instructors, and the nature and frequency of instructors' feedback are assessed using both Visual Analogue Scales (VAS) and open-ended questions.

**Results:** Among the 22 VAS items, the Cronbach's Alpha of the survey is 0.89. All survey participants (N=62) responded that the most effective mode of learning is "to use 3D models" (mean=81.5). According to the survey results, the least effective mode of learning is "demonstration by a classmate" (mean=51.2). When comparing the perceptions of third with fourth year students, there were statistically significant differences (using independent t-tests), suggesting that the interactions with instructors in the pre-clinic learning were perceived differently between the two groups. Content analysis of the open-ended questions led us to further interpret the quantitative data analysis results.

**Conclusions:** Among existing literature, very little is known regarding the learning that occurs in the pre-clinical setting. Our findings on third and fourth year students' different perceptions toward effective learning in clinical simulation provides a new understanding in simulation teaching and learning and can help inform UBC's DMD program renewal effort.

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## 12. **MicroRNAs as Potential Novel Classifiers of Oral Premalignant Lesions**

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**Objectives:** Oral Squamous Cell Carcinoma (OSCC) is a worldwide problem, with a dismal 5-year survival rate (~50%). A better understanding of the molecular basis of OSCC—with particular emphasis on disease initiation and progression—is needed to improve clinical outcomes. To date, the role of microRNAs (miRNAs), a class of short, non-coding, single-stranded RNAs that negatively regulate gene expression, in oral tumourigenesis and oral premalignant lesions (OPLs) specifically, is largely unknown. The objective of this study was to identify miRNAs that are deregulated in early OPLs and to select those with the greatest potential as future biomarkers.

**Methods:** For each of ten patient cases, we examined miRNA expression in histologically different OPL biopsies obtained from a single, contiguous field. Diseased regions in the oral cavity were detected by either white light and/or a hand-held Fluorescence Visualization device in the operating room. Total RNA was isolated from each microdissected specimen and profiled for the expression of 742 human miRNAs using quantitative real-time polymerase chain reaction. Data were normalized based on manufacturer guidelines and a minimum of two-fold expression change relative to matched normal was used to define candidate miRNAs.

**Results:** We identified several miRNAs that are aberrantly expressed at the earliest stages of oral tumourigenesis. The most frequently upregulated and downregulated miRNAs were miR-21 and miR-375, respectively. Both of these, and some of the other detected signature miRNAs, are widely mentioned in the literature and it has been suggested that they play crucial roles in the oncogenic pathways of multiple malignancies.

**Conclusions:** Molecular characterization of intralesional progression of the altered oral field allows for the delineation of key genetic events driving OSCC progression. This is critical for improved disease management. Ultimately, our data will aid in the development of a novel genomic platform for risk assessment, diagnosis and novel targeted therapies.

**Acknowledgements:** This work is supported by the Canadian Cancer Society, the Pacific Otolaryngology Foundation, and the Canadian Institutes of Health Research.

**13. Can Radiographic Features Predict the Recurrence of Keratocystic Odontogenic Tumours?**

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**Objectives:** The high recurrence rate (2-62%, average 30%) of keratocystic odontogenic tumours (KCOT) means that it would be desirable to find some clinical features that could predict recurrence and enhance the management of such cases. Currently there is a lack of comprehensive literature on the difference between recurrent KCOT (rKCOT) and non-recurrent KCOT (nrKCOT), especially in radiographic features. The objective of this study was to compare radiographic features between rKCOT and nrKCOT cases.

**Methods:** Using key words to search the pathology database of the BC Oral Biopsy Service, we identified 117 odontogenic keratocysts from 2000 to 2009. After excluding the orthokeratinized odontogenic keratocyst, Naevoid basal cell carcinoma syndrome (NBCCS) associated KCOT, and multiple KCOT, 64 cases (34 rKCOTs; 30 nrKCOTs) with at least 5-year follow-up were included in this study. We were able to retrieve panoramic films or digital images for a subgroup of these cases (20 rKCOTs; 15 nrKCOTs). All of the radiographs were reviewed (DM) without knowing the outcome. We collected demographics, the lesional location and the treatment information, and examined the key radiographic features including size, shape and relationship to the surrounding morphological structures.

**Results:** There is no statistically significant difference between the two groups in age, gender, distribution on maxilla or mandible or and surgical manors. In radiographic characteristics, there is no difference between the rKCOT group and the nrKCOT group in size, shape, margin characteristics, or relationship to the surrounding structures. Although the difference is not statistically significant, the rKCOT cases show a slight increase in the premolar location (73% vs. 50%) and with the larger size (> 2 dental units; 70% vs. 52%).

**Conclusions:** The radiographic features cannot predict the recurrent risk of a solitary KCOT.

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#### 14. **Dental Education Curricular Renewal: a Large-Scale Literature Review**

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**Objectives:** To determine the characteristics of current dental education, we employed a large-scale literature review as one component of a larger study aimed at dental curriculum renewal, in order to identify problems and current curricular and pedagogical developments.

**Methods:** The authors reviewed all of the abstracts from articles published over the past ten years, 2001 to 2011, by the *Journal of Dental Education*, the *European Journal of Dental Education*, and the *Journal of the Canadian Dental Association*. Studies that were related to the following topics were selected: dental curriculum, problems and/or improvements in preclinical or clinical dental education ( $N_{\text{dental curriculum}}=1250$ ,  $N_{\text{problems,improvementpreclinic}}=402$ ,  $N_{\text{clinical}}=631$ ). Out of this literature, 44 articles fit the scope of our inquiry and thus were used for this review.

**Results:** Among the current literature reporting on newly developed curricula emerging from Norway, China, Arizona, and California, there seems to be a common trend to seek to achieve the following goals: (i) reducing unnecessary redundancy and overlap between courses; (ii) providing clinical experience to students earlier in the curriculum; and (iii) providing lectures in a more integrated manner. Generally, many dental schools seem to have focused on effective integration of their curricula and maximum clinical exposure for students in the process of making improvements in their programs.

**Conclusions:** Various attempts and suggestions have been made in the dental curriculum renewal literature and we conclude that there is a need to understand which curricular concerns are unique to the Canadian dental education community. We need to further explore the contribution of lessons learned from dental schools in other countries in adapting some of these suggested curricular renewal strategies in UBC's DMD Program specifically or to any other Canadian dental school in general.

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**15. Oral Health Disparities in Vancouver's Downtown Eastside**

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**Objectives:** Vancouver's Downtown Eastside (DTES) is one of the poorest locales within Canada. Our previous data have demonstrated that the DTES is at high risk for oral cancer due to its high-risk behaviours and high incidence (1:150 screened dental patients vs. 1:10,000 in BC/Canada). This study sought to determine the availability of dental coverage, access to dental care, and oral health status in this community, and to compare this with the newly published national data from the Canadian Health Measures Survey (CHMS).

**Methods:** Mobile screening clinics were established at three main community centres: Vancouver Native Health Society; Women's Centre; and LifeSkills Centre. Eligibilities included those of age 18 or over and residing in the DTES for at least 3 months. Data were collected through personal interviews with survey questionnaires. Oral health status was obtained through examination by a dentist and an oral pathologist.

**Results:** From four screening clinics, 106 participants were screened. Comparing to CHMS data, the DTES has more Aboriginals, and low-incomers are less educated. Less than half of the participants used any dental services within the past 12 months (49% vs. 74%,  $P < 0.0001$ ). Many (71%) of them have limited government dental coverage; yet, the responses show cost and less priority are the barriers. There was a drastic increase in DMT scores (19.72 vs. 2.72,  $P < 0.0001$ ). Participants have a lower number of teeth present (17.38 vs. 24.53,  $P < 0.001$ ) and poorer oral hygiene (brushing  $< 2$  times/day, and flossing  $< 5$  times/week,  $P < 0.05$ ). The presence of mucosal lesions was similar to those in low-income groups, but significantly higher than the general group ( $P = 0.02$ ).

**Conclusions:** Compared to CHMS data, this community is dentally underserved with a prevalence of oral health problems. Facilitating the use of existing limited resources, and raising awareness, may improve oral health in this community.

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## 16. Who's Checking Daily Oral Healthcare in Long-Term Care?

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**Background:** Poor oral health in long-term care (LTC) facilities is rampant; however, we do not have an effective strategy to improve LTC residents' daily oral healthcare (DOH). In British Columbia, the government has regulation BC Reg 96/09, which specifies the joint responsibility of dental professionals and LTC administrators to maintain the DOH of people in LTC facilities. More than a decade after this regulation appeared, conflicting priorities hinder this care.

**Objectives:** To answer the question of how the government's regulation impacts DOH in LTC facilities.

**Methods:** A secondary analysis was conducted of open-ended interviews with 14 LTC administrators before the regulation was implemented<sup>1</sup>. Subsequently, we conducted similar interviews with five government officials and five administrators to explore how the regulation was developed and implemented. Participants for interviews were selected purposefully to obtain a broad perspective on our objective. We used a constant comparison technique to analyze relationships between the various perspectives and the trustworthiness of the analysis was determined by triangulating with publications and participants' validation.

**Results:** Administrators emphasized that, before the regulation was introduced, there was a need for constant reminders, continuing education and administrative accountability to maintain DOH. The government officials developed the regulation to provide for a clinical examination annually by a dental professional for every facility resident. Administrators do not implement the regulation as expected because they do not expect government officials to inspect DOH. The government inspectors do not enquire about oral healthcare unless there is a written record from dental professionals for treatment of specific mouth problems.

**Conclusions:** The regulation to manage oral healthcare in LTC facilities is not being implemented as anticipated because of inadequate collaboration between dental professionals, administrators, and government inspectors.

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<sup>1</sup> MacEntee MI, Thorne S, Kazanjian A (1999). Conflicting priorities: oral health in long-term care. *Spec Care Dentist* 19:164-172.



## 17. The Role of Src Family Kinases on Palatal Fusion

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**Objectives:** Src family kinases (SRK) are involved in the regulation of cell–cell adhesion through up regulation of E-cadherin endocytosis. SRK can exert their effect as a downstream signalling effector for TGF- $\beta$ , which plays a pivotal role in palatal fusion. The involvement of SRK during palatal fusion has not been examined. In the present study, we investigated the effect of PP2, an SRK specific inhibitor, on palatal fusion.

**Methods:** Immunofluorescence analysis was performed to investigate SRK during palatal fusion. Palatal organ culture was conducted to investigate the effect of PP2 on palatal fusion. The effect of PP2 on E-cadherin distribution was evaluated by immunofluorescence. The regulation of SRK was investigated in palatal tissues from TGF- $\beta$ 3 null mice and immunofluorescence.

**Results:** SRK activity was localized at cell–cell junctions in the medial edge epithelium (MEE) and increased just before adhesion with a peak at 30 h of organ culture that returned to the basal level at 48 h. PP2 caused the failure of palatal fusion due to an adhesion defect in the anterior and middle palatal regions. In control palatal tissue, E-cadherin accumulated at the apical side of the MEE with variously-sized E-cadherin positive vesicles in the cytoplasm before adhesion. In the experimental group, the MEE cell surface was ubiquitously surrounded by tubular-like structures with strong E-cadherin signals. However, E-cadherin signals were observed in neither the cytoplasm nor the nucleus. In TGF- $\beta$ 3 null mice, SRK activity was completely inhibited and restored by exogenous addition of recombinant TGF- $\beta$ 3.

**Conclusions:** Our data indicates that SRK may act as a downstream effector for TGF- $\beta$ 3 and play a pivotal role in palatal adhesion through the regulation of E-cadherin degradation.

**Acknowledgements:** This work was supported by NIDCR grant R01 DE16296.

**18. Novel *Ex Vivo* Biofilm Model: Comparative Root Canal Disinfection Study**

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**Objectives:** Bacteria, organized as biofilms within the root canal, can cause apical periodontitis (AP). Adequate removal of the microorganisms from the root canal is essential for the prevention and treatment of AP. While difficulties in disinfection have been recognized, limited data are available to directly assess the effectiveness of bacteria removal by treatment. We sought to develop a standardized *ex vivo* biofilm model, closely resembling the *in vivo* clinical situation, to quantify and compare the efficacy of hand, rotary nickel–titanium (NiTi) and self-adjusting file (SAF) instrumentation in the removal of biofilm bacteria.

**Methods:** Thirty-six extracted single-rooted teeth with oblong canals were selected. Each tooth was split longitudinally and a 0.2 mm-wide groove was placed in the apical 2 to 5 mm of the canal. After growing mixed bacteria biofilm inside the canal under anaerobic conditions, the split halves were reassembled in a custom block, creating apical vapour lock. Teeth were randomly divided into three treatment groups (n = 10 per group) using: (1) hand K-file; (2) ProFile NiTi rotary; and (3) SAF. Irrigation consisted of 10 mL 3% NaOCl and 4 mL 17% EDTA. Six teeth received no treatment. Areas inside and outside the groove were examined using a scanning electron microscope.

**Results:** Within the groove, a smaller area remained occupied by bacteria after the use of SAF than ProFile or K-file (3.25%; 19.25%; 26.98%) ( $P < 0.05$ ). For all groups, significantly more bacteria were removed outside the groove than inside ( $P < 0.05$ ). No statistical differences were found outside the groove ( $P > 0.05$ ).

**Conclusions:** Although all techniques equally removed bacteria outside the groove, SAF significantly reduced more bacteria from the apical groove. No technique was able to completely remove the bacteria. The biofilm model represents a potentially useful tool for the future study of root canal disinfection.

**Acknowledgements:** Supported in part by the Canadian Academy of Endodontology and the American Association of Endodontists.

## 19. Production and Purification of Cathepsin V Mutant by Fermentation

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**Objectives:** Human cathepsin V, a potent elastin-degrading protease, shares a 77% amino acid sequence identity with cathepsin L, which exhibits only minimal elastolytic activity. Previous evaluation of cathepsin V/L chimera variants revealed that two exosites contributed significantly to the elastolytic activity of cathepsin V. An elastolytically inactive cathepsin V mutant M12 was constructed with a replacement of either exosites with the analogous residues present in cathepsin L. We sought to produce a highly purified cathepsin mutant in a large quantity for future crystallography study.

**Methods:** A cathepsin V M12 mutant pPIC9 expression vector was transformed into the GS115 strain of *Pichia pastoris*, and a clone expressing high levels of the cathepsin V was selected for fermentation. During fermentation, a 3-hour glycerol-fed batch was performed at 46 hours after inoculation, methanol induction was initiated at an OD<sub>600</sub> of 44, and harvesting was carried out 96 hours later. The expression level of the enzyme was monitored by its Z-FR-MCA hydrolyzing activity. The five-litre supernatant of pro-cathepsin V was concentrated, the pro-enzyme solution was activated with 0.6 mg/mL pepsin at 37°C for 30 minutes, and then the activated enzyme was purified by Fast Protein Liquid Chromatography.

**Results:** Approximately 103 mg of crude pro-cathepsin V was produced. The pro-enzyme was activated, purified and concentrated to 2.046 mg/mL. Its active site concentration was determined by E-64 titration to a final concentration of 370 μM. However, SDS-PAGE analysis of the column-purified material revealed a large protein smear at a molecular range of 35-55 kD, indicating hyperglycosylation of the product. Mature cathepsin V has two putative N-glycosylation sites.

**Conclusions:** The fermentation process yielded a very high amount of active cathepsin V. However, heterogenous glycosylation of the cathepsins at its two putative glycosylation sites may affect crystallization efforts. We will delete both glycosylation sites by site-directed mutagenesis.

**Acknowledgements:** This research was supported by a UBC Faculty of Dentistry Undergraduate Student Summer Research Award. The authors would like to thank Raymond Pan for his instruction and comments on protein purification.

## 20. **Clinical and Molecular Profiles of Different-Aged Oral Cancer Patients**

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**Introduction:** Oral squamous cell carcinoma (OSCC) was a common disease of heavy-smoking elderly; however, the incidence of OSCC in younger patients is increasing worldwide. Research to understand why could impact oral cancer control.

**Objectives:** (1) Determine the characteristics of OSCC in different age-groups within the BC Oral Cancer Prevention Longitudinal (OCPL) study. (2) Determine molecular profiles of OSCC among groups.

**Methods:** From 1995 to 2011, we recruited 479 oral cancer patients. These were categorized into three age groups based on age at initial diagnosis: *young group* ( $\leq 45$  yrs) consisting of 14% of patients (64); *conventional group* (46-75 yrs) 73% (352); and *old group* ( $> 75$  yrs) 13% (63). The following information was collected: demographics and smoking habits of the patients, clinicopathological features, treatment, and outcome of OSCC. Of these patients, 13% (64) of cases were analyzed for loss of heterozygosity (LOH) at chromosome arms 3p, 4q, 8p, 9p, 11q, 13q, and 17p.

**Results:** OSCC in *young* was found to occur more in non-smokers (60% vs. 26% in *conventional* and 37% in *old*, both  $P < 0.05$ ); be on the tongue (84% vs. 47% and 46%, both  $P < 0.05$ ); be small at diagnosis (T1/T2 95% vs. 83% and 81%, both  $P < 0.05$ ); recur less as invasive cancer (6% vs. 25% and 24%, both  $P < 0.05$ ); and less death from the disease (13% vs. 27% and 35%, both  $P < 0.05$ ). For LOH, a difference was only noted at 3p14 (containing *FHIT*). *Young* had a higher 3pLOH (100% vs. 67% in *conventional*,  $P = 0.049$  and 50% in *old*,  $P = 0.03$ ). There were no differences in the other parameters among the three age groups.

**Conclusions:** Non-smokers, tongue location, better prognosis, and LOH at 3p14 characterize the *young group*. We are conducting further molecular studies.

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## 21. **Syndromic Keratocystic Odontogenic Tumours**

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**Introduction:** The keratocystic odontogenic tumour (KCOT) is a major feature of nevroid basal cell carcinoma syndrome (NBCCS).

**Objectives:** (1) The clinical and radiological presentations of the syndromic KCOT at first presentation. (2) Their long-term response to treatment. (3) To compare these findings with those of cases of solitary KCOTs (DMFR 2010:39:167-175) occurring in the same community during a similar period of time.

**Methods:** A consecutive series of syndromic cases of KCOTs was identified in dental records from the University of Hong Kong. The first presentation of the cases occurred between 1986 and 2000. They were followed-up.

**Results:** Six cases of syndromic KCOTs were found; four were female and two male. The mean age at first presentation was  $22.25 \pm \text{SD } 6.18$  years. One case was discovered as an incidental finding. Three of the others were discovered following purulent nasal discharge. Five presented with at least two KCOTs at the time of first presentation or shortly afterwards. Both were in the mandible in one case, both were in the maxilla in one case, and there was one in each jaw for the remaining. Computed tomography was prescribed for only one case at first presentation. Four cases exhibited a recurrence after a mean interval of  $5.5 \pm \text{SD } 1.7$  years after primary surgery. The first clinical feature which led to a diagnosis of NBCCS in each case was the KCOT.

**Conclusions:** These NBCCS cases differed from the cases of solitary KCOTs occurring in the same community during a similar period of time. The NBCCS cases first presented at a significantly younger mean age. Furthermore, at least one treated KCOT in a NBCCS case was significantly more likely to recur. Diagnosis of a KCOT, particularly in a young patient, should lead to consideration that this patient may be syndromic. Such cases should be subject to long-term follow-up.

## 22. **Role of Fibroblast Phenotype and Pericellular Matrix in Wound Healing**

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**Objectives:** Scar formation as a result of wound healing in skin is associated with an increased deposition of extracellular matrix (ECM) by fibroblasts. Wounds in the skin heal with scar formation while in the human oral mucosa (OM) they heal without scars. Thus, comparing fibroblast functions in these two tissues may provide novel information about the factors that regulate scar formation. The ECM niche that surrounds tissue cells has a profound effect on their function and differentiation. Therefore, we hypothesized that ECM produced by skin and OM fibroblasts are inherently distinct and determine the wound-healing outcome. To this end, we compared gene expression of human fibroblast lines from OM and skin and analyzed their response to three-dimensional (3D) ECM substrates generated by these cells *in vitro*.

**Methods:** Primary fibroblasts from five parallel cell lines isolated from human skin (breast) and OM (attached gingiva) were cultured in high density up to 14 days, and expression of key genes involved in wound healing and scar formation was analyzed by real-time PCR. To determine the role of the ECM on cell function, gingival and skin fibroblasts were seeded on gingival or skin fibroblast-derived 3D ECM, or on type-I collagen in various combinations, and gene expression was analyzed.

**Results:** Gingival and skin fibroblasts showed distinct expression of several genes involved in wound healing including growth factors, ECM proteins and matrix metalloproteinases. Skin fibroblasts produced significantly more protein relative to gingival fibroblasts on the 3D ECM substrates, and these substrates regulated skin and gingival fibroblast gene expression differently.

**Conclusions:** Gingival and skin fibroblasts have distinct gene expression profiles and ECM niches generated by these cells regulate their phenotypes differently. Distinct phenotypes of fibroblasts and composition of the ECM produced by these cells may contribute to the different wound healing outcomes in skin and OM.

**Acknowledgements:** Supported by the Canadian Institutes of Health Research (CIHR) and the CIHR Skin Research Training Centre.

**23. Oral Appliance Treatment of Obstructive Sleep Apnea: Long-Term Side Effects**

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**Objectives:** Obstructive sleep apnea (OSA) often leads to poor sleep quality, daytime sleepiness, increased motor vehicle accidents, hypertension, or stroke. Oral appliances that hold the mandible in a forward position and enlarge the airways during sleep have increasingly been used to treat OSA. Though well tolerated, oral appliances have known side-effects, the most common being occlusal changes. As OSA treatment continues throughout a patient's lifetime, a long-term examination of these side-effects is warranted. Therefore, the objective of this study was to evaluate dental changes in OSA patients after at least eight years of oral appliance therapy.

**Methods:** In this retrospective study, orthodontic study models of patients with a minimum of eight years of appliance wear were collected. Models were measured with a digital caliper, and values assessed included overjet, overbite, crowding, canine and molar position, intermolar and intercanine distances. Method errors were calculated using Dahlberg's formula. Descriptive measures and paired student t-tests were used to analyze the data.

**Results:** A total of 77 patients (average age at start of treatment:  $47.5 \pm 10.2$  years, 62 males) were included in this study. The average treatment length was  $11.1 \pm 2.8$  years. Method error for dental measurements ranged from 0.13 to 0.64 mm. There was a significant correlation ( $p < 0.001$ ) between time under treatment and the decrease in both overjet and overbite ( $r^2 = 0.10$  and  $r^2 = 0.07$ , respectively). Over the period evaluated, there was a significant ( $p < 0.001$ ) reduction in the overbite ( $2.3 \pm 1.6$  mm), overjet ( $1.9 \pm 1.9$  mm), and mandibular crowding ( $1.3 \pm 1.8$  mm). A corresponding significant ( $p < 0.001$ ) increase of mandibular intercanine ( $0.7 \pm 1.5$  mm) and intermolar ( $1.1 \pm 1.4$  mm) width was observed. Significant anterior movement in canine and molar position of the lower arch relative to the upper arch was also observed.

**Conclusions:** Side-effects of oral appliances appear to continue over time, producing clinically relevant changes to the occlusion and dental arches.

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**24. Acceptance of Innovative Caries Management in a Community Dental Clinic**

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**Objectives:** “Project Smile-Aid” (PS-A) pilots a pro-active approach to caries management in a dental public health setting. Motivational-style caregiver counselling, interim therapeutic restorations (ITRs) and topical remineralization agents are combined (“intervention”). The overall aims of the research are to determine “intervention” feasibility, its acceptance by immigrant parents and its impact on child oral health-related quality of life. Issues related to acceptance of the “intervention” by both parents and clinic staff will be the focus of this presentation.

**Methods:** Caries-active pre- and school-aged children from culturally-diverse, low-income families were recruited at a community dental public health clinic in Vancouver, Canada. Target sample size was 50. Baseline measures [dental health status, oral hygiene, child’s behaviour, and Early Childhood Oral Health Impact Survey (ECOHIS) scores] were compared with measures at post-“intervention” intervals. One calibrated clinician recorded baseline measures and delivered the “intervention.” All instruments, supplies and overhead costs, including dental assisting, were grant-funded. To enhance the support of clinical staff for this departure from customary caries management, staff were consulted during all planning phases. An interactive “in-service” presentation was held prior to the project launch.

**Results:** To date, 21 eligible parent-child dyads have been approached, have agreed to participate, and the “intervention” has been delivered to all 21 children [mean age 2.8 years]. Placement of multiple ITRs in active, pre-cooperative children is a significant challenge. Despite the challenges of child behaviour, parental satisfaction with their child’s response 48 hours post-“intervention” was overwhelmingly positive. Clinic-related issues (e.g. operatory availability) remain major obstacles to delivering the “intervention” in this public setting.

**Conclusions:** Low-income, immigrant parents are eager to participate in stabilizing their child’s caries when wait-times for definitive treatment are lengthy. However, operational issues and overcoming staff skepticism challenge innovation in customary caries management protocols.

**Acknowledgements:** Supported by a 2010 UBC Faculty of Dentistry Pilot Project Grant to KMC.



25. **Kinetic Characterization of a Novel Class of Anti-Collagenase Cathepsin-K Inhibitors**

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**Objectives:** Osteoporosis is a bone degenerative disease caused by excessive osteoclast activity. cathepsin K is a cysteine protease that is highly expressed in osteoclasts. This enzyme forms a complex with chondroitin 4-sulphate (C4-S) creating collagenase activity. The objective of the research was to determine the kinetic parameters of collagen hydrolysis by cathepsin K and the evaluation of the potency of previously identified cathepsin K/C4-S complex formation inhibitors.

**Methods:**  $K_m$  and  $k_{cat}$  values were determined for the degradation of type-I collagen by the cathepsin K/C4-S complex. Michaelis-Menten kinetics and a Lineweaver-Burk analysis were used to evaluate the data. Structurally-related putative cathepsin K/C4-S complex formation inhibitors partially identified from a chemical drug library were evaluated for their ability to prevent cathepsin K/C4-S-mediated degradation of collagen in a non-active site inhibitory mechanism. An  $IC_{50}$  was determined for each effective compound, and the accessibility of the active site of cathepsin K was determined using a synthetic fluorogenic peptide substrate, Z-FR-MCA.

**Results:** The kinetic values for cathepsin K/C4-S-mediated degradation of collagen are as follows:  $K_m = 1.6 \mu M$ ,  $k_{cat} = 6 h^{-1}$  and  $k_{cat}/K_m = 1,062 M^{-1}s^{-1}$ . Six out of the 8 putative cathepsin K inhibitors were able to prevent the degradation of collagen: Aurintricarboxylic Acid ( $IC_{50} = 9.6 \mu M$ ); Ellipticine ( $IC_{50} = \sim 100 \mu M$ ); Epigallocatechin gallate ( $IC_{50} = 52 \mu M$ ); Raloxifene ( $IC_{50} = 85 \mu M$ ); Suramin ( $IC_{50} = 6.7 \mu M$ ); and Tamoxifen ( $IC_{50} = \sim 150 \mu M$ ). All but Suramin appeared to be non-active site inhibitors as the degradation of Z-FR-MCA was not impaired in the presence of the inhibitors.

**Conclusions:** Degradation of collagen by the cathepsin K/C4-S complex can be inhibited by structurally-related compounds in a non-active site related manner. Interestingly, Tamoxifen and Raloxifene have been clinically demonstrated to inhibit bone resorption via an estrogen related pathway.

**Acknowledgements:** This research was supported by a UBC Faculty of Dentistry Undergraduate Student Summer Research Award.

**26. Ultrastructural and Biochemical Approaches to Evaluate Type I Collagen Degradation**

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**Objectives:** Aberrant degradation of triple helical type I collagen by cathepsin K, which mediates bone resorption by forming complexes with chondroitin 4-sulfate (C4-S) or dermatan sulfate (DS), is a key feature of osteoporosis. Therefore, a detailed investigation of cathepsin K-GAGs complexes in degradation of type I collagen is essential. In this study, we examined the degradation mechanism and cleavage sites of type I collagen derived from cathepsin K/C4-S or DS.

**Methods:** The enzymatic degradation of type I collagen fibres of rat tail tendons by cathepsin K was investigated by high-resolution scanning electron microscopy (SEM). The regulation of collagen I degradation by GAGs was also envisaged by SEM. However, a direct approach to identifying cathepsin K cleavage sites in triple helical type I collagen to generate potential neoepitope antibodies has been undertaken. N-terminal sequencing was used to identify novel neoepitopes generated by cathepsin K derived degradation of mouse type I collagen.

**Results:** SEM revealed a progressive degradation of type I collagen fibrils, becoming shorter and thinner based on their incubation time. In addition, surface morphologies also showed that attacks by cathepsin K in the presence of GAGs resulted in uniform granule penetration at certain places on the surface. Sequencing data confirmed the initial cleavage sites after NTX in triple helix and antibodies specific for the different neoepitopes generated by such cleavage events are in progress.

**Conclusions:** This study describes how scanning electron microscopy is useful in demonstrating the binding of cathepsin K-GAGs complexes on type I collagen and in their degradation. These findings will also help to explain the specificity of neoepitope antibodies as biomarkers in bone resorption.

**27. Evaluation of Ultrasonic Irrigation Systems for Root Canal Debris Removal**

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**Objectives:** The goal of irrigation is to remove pulp tissue and/or microorganisms from the root canal system as well as to remove the smear layer and dentin debris that are produced by instrumentation of the root canal. The aim of this study was to evaluate two continuous-flow ultrasonic irrigation systems in straight and curved root canals.

**Methods:** Twenty-four maxillary, recently-extracted anterior teeth of curvature less than 10 degrees, and 24 mesial roots of mandibular molars with a curvature between 15-30 degrees, were instrumented to size 40, 0.04 taper and 35, 0.04 taper. The teeth were divided into three experimental groups according to the final irrigation: conventional syringe irrigation with 30-gauge side-vented needle; the PiezoFlow ultrasonic irrigation system; and the VPro StreamClean ultrasonic irrigation system. In all experimental groups, 15 mL of 3% sodium hypochlorite was used after instrumentation. Both ultrasonic systems were set at a flow rate of 15 mL/min and used for 1 minute at the ultrasonic power setting recommended by the manufacturer. This was followed by 3 mL of 17% EDTA for 2 minutes and 2 mL of sterile water. Scanning electron microscope images were acquired of the apical region (1, 3 and 5 mm) at a magnification of 200 × for debris and 1000 × to assess the smear layer.

**Results:** In the anterior, straight teeth in all three groups left more debris at the 1 mm area compared to the 3 mm area and the 3 mm area left more debris than the 5 mm area. There was less debris remaining in all three areas (1, 3 and 5 mm) with both ultrasonic systems.

**Conclusions:** The use of continuous-flow ultrasonic systems left less debris in the apical region of straight anterior teeth than conventional syringe irrigation with a side-vented needle.

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**28. Measurement of Apical Pressure Using Positive and Negative Pressure Irrigation**

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**Objectives:** This study measured the pressures generated during positive and negative pressure irrigation at the periapex of an *in vitro* tooth model using a novel method of measurement. We also investigated the effect of flow rate, depth of needle placement, and needle design. Apical pressure was correlated with the extent of dye clearance during irrigation in a similar model.

**Methods:** The mesiobuccal canal of a mandibular molar was prepared to apical size 35 and 0.06 taper. The tooth was placed into a custom testing fixture and coupled to a pressure transducer and oscilloscope. Three irrigation needles were used: a 30-gauge open-end needle with a flexible polyimide tubing tip; a 30-gauge closed-end side port needle; and an ISO 32 closed-end needle with 12 radial microholes in the distal 0.7 mm. A pump was used to deliver irrigant at precise flow rates at 5 mm, 3 mm and 1 mm from the working length. A plastic root canal model filled with dye was used to measure the extent of dye clearance beyond the needle tip.

**Results:** Positive pressure irrigation revealed an increase in apical pressure ( $P < 0.001$ ) dependent on both the flow rate and the depth of needle placement. Needle designs with safety features yielded statistically significant lower apical pressures than the open-end needle ( $P > 0.001$ ). There was no further clearance of dye beyond the needle tip past a flow rate range of 5-6 mL/min. Negative pressure irrigation revealed negative apical pressures, regardless of needle design, flow rate or depth of needle placement.

**Conclusions:** Irrigation needles with safety design features generate lower apical pressures during positive pressure irrigation. Irrigation beyond 5-6 mL/min may not confer additional benefits in irrigation effectiveness during positive pressure irrigation. Negative pressure irrigation confers a major safety benefit to patients.

**Acknowledgements:** Supported by the Canadian Academy of Endodontics and the American Association of Endodontists.

## 29. **The Role of Pannexin-3 in the Formation of Intramembranous Bone**

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**Objectives:** Pannexin-3 is one of three pannexin hemichannels but is the only one expressed in skeletal tissues. Researchers have shown that PANX3 functions in endochondral ossification to regulate a switch in chondrocyte fate from proliferation to differentiation. The aim of this project was to determine whether PANX3 is involved in the commitment of ectomesenchymal cells to an osteogenic fate in intramembranous bones of the face.

**Methods:** Several avian retroviruses were cloned as follows: full-length chicken *PANX3*; deletion of the C-terminal leucine zipper; and interference-RNA construct to target gallus *PANX3*. The control virus was expressing *GFP*. The viruses were injected into the maxillary prominence of 2.5-day chicken embryos. These embryos were re-incubated for 12 days to permit full ossification of facial bones. The skulls were then stained in wholmount and the sizes and positions of intramembranous bones assessed via 3D scanning using the optical projection tomography scanner. Statistical analysis of size and shape changes in individual palatine bones was carried out.

**Anticipated Results:** We predict that wild-type *PANX3* is sufficient to induce an increase in the size of bones or to produce ectopic ossification. We anticipate that deletion of the leucine zipper will lead to a dominant negative effect or loss-of-function of *PANX3*. The sh*PANX3* is predicted to reduce the expression of endogenous *PANX3* and lead to an inhibition of bone formation. Thus far, external analysis of the embryos expressing the leucine zipper deletion shows an upper beak deviation which is likely due to an underlying bone differentiation phenotype. Further results will be presented for all of the different viruses.

**Anticipated Conclusions:** From these different viral constructs, we will determine whether PANX3 is required or sufficient for the commitment of ectomesenchymal cells to an osteogenic fate.

**Acknowledgements:** The authors would like to thank the Richman lab and Steve Bond from the Naus lab for their immense help and contribution. This investigation is supported in part by a summer research studentship from CIHR and CIHR operating grants to JMR.

**30. Mechanism of RAW 264.7 Macrophage-Mediated Depletion of Hydrogen Peroxide**  
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**Objectives:** The macrophage is capable of producing hydrogen peroxide ( $H_2O_2$ ), a chemical compound integral to its immunological killing function.  $H_2O_2$  is a highly toxic compound and, as such, the macrophage also needs to be able to inactivate this molecule for its own survival. While it was previously found that the exogenously-added  $H_2O_2$  could be depleted by the RAW 264.7 cell line, the mechanism of depletion remains unknown. This study was conducted to elucidate the mechanism of  $H_2O_2$  depletion by the RAW 264.7 cell line.

**Methods:** Initially, 0.5 mL of  $1 \times 10^6$  cells/mL of RAW 264.7 cells was added to each well of a 96-well plate. The cells were then incubated for 24 hours at  $37^\circ C$  in the presence of three different inhibitors: 50 mM 3-amino-1,2,4-triazole (3AT), a catalase inhibitor; 20 mM mercaptosuccinic acid (MSA), a glutathione peroxidase inhibitor; and 200  $\mu M$  L-buthionine-(S,R)-sulfoximine (BSO), a synthesis inhibitor of the antioxidant-reduced glutathione. Subsequently, each well was washed with phosphate-buffered saline (PBS). Cells were then exposed to 40  $\mu M$   $H_2O_2$ . After 15 and 30 minutes, intracellular and extracellular  $H_2O_2$  concentrations were quantified with the fluorescence-based Amplex Red reagent kit.

**Results:** Cells incubated with 3AT for 24 hours showed significantly increased fluorescence extracellularly, both at 15 and 30 minutes after the addition of 40  $\mu M$   $H_2O_2$ . In contrast, MSA treatment showed significantly higher fluorescence intracellularly at 15 and 30 minutes after  $H_2O_2$  addition. Incubation with BSO had no noticeable effect on the fluorescence intensity.

**Conclusions:** Exhaustion of exogenous  $H_2O_2$  by the RAW 264.7 cell line involves both catalase and glutathione peroxidase. Extracellularly, it is depleted by catalases that are released from cells, whereas intracellularly, it is depleted by the combined action of both catalase and glutathione peroxidase following the influx via diffusion.

**Acknowledgements:** The authors would like to thank the UBC Faculty of Dentistry for generous support and Haisle Moon for her technical support. This study was funded by a CIHR Health Professional Student Research Award.

### 31. Challenges in Establishing Primary Oral Squamous Cell Carcinoma Cell Lines

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**Objectives:** Due to the lack of *in vitro* and *in vivo* models of oral carcinogenesis, studies must depend on fresh-frozen oral cancer tissues for experimentation. Cell lines that are representative of particular cancers provide an enriched resource to study molecular changes involved in tumourigenesis. Here we describe the challenges encountered in the establishment of oral cancer cell lines derived from patients with oral squamous cell carcinoma (OSCC).

**Methods:** Cell lines were derived from nine surgically resected tissue specimens from three OSCC patients. Tissues were minced and suspended in  $\alpha$ -MEM containing 10% (v/v) FBS, 200 iu/mL penicillin, 200  $\mu$ g/mL streptomycin, 2.5  $\mu$ g/mL amphotericin B, 0.4 ng/mL EGF, 2  $\mu$ g/mL hydrocortisone and 2 mM L-glutamine, and seeded into 10 mm tissue culture dishes. All cultures were incubated in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C.

**Results:** The early outcome of these cultures did not reflect the clinical characteristics of the tumour of origin. Bacterial and/or yeast contamination was seen in two of the specimens. Two additional tissue specimens did not show any evidence of cell outgrowth while three tissues showed overgrowth of host fibroblasts even after partial trypsinization. One sample was characterized by limited epithelial growth with senescence at passage 3. Continuous cell growth showing epidermoid cell-like appearance was seen in one tissue sample. The epithelial origin of the culture was established by showing the expression of epithelial cell surface markers such as EMA and cytokeratins.

**Conclusions:** Although it is quite challenging to establish a cancer cell line from primary tissue, especially from the oral cavity, these cell lines can serve as useful molecular tools in unraveling the regulatory pathways in oral cancer and lead to a better understanding of oral cancer biology.

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### 32. **Oral Health in Inner-City School-Aged Children**

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**Objectives:** Oral health in inner-city Vancouver school-aged children has not been studied. Our objectives were to: (1) describe oral health, oral hygiene and treatment needs in inner-city Vancouver elementary school-aged children; and (2) examine differences in oral health between gender and ethnic groups.

**Methods:** Principals of all of the elementary schools in inner-city Vancouver were approached and the aims of the study were explained to them. Seven out of 10 principals agreed to participate and consent forms were sent to parents/guardians. The clinical data included examinations of oral health and digital assessments of oral hygiene. Self-reported data comprised information about ethnicity, gender and place of birth.

**Results:** A total of 462 children from different age groups were examined (kindergarten and grades 1-7). Children were primarily from three main ethnic groups: Filipino (20%), Indian (18%) and Vietnamese (14%). Almost 30% of examined children were born outside of Canada. When oral health in the permanent dentition was compared, girls had a higher mean  $\pm$  SD number of both caries surfaces ( $0.60 \pm 1.3$ ) and filled surfaces ( $0.85 \pm 2.4$ ) than boys did ( $0.31 \pm 0.9$  and  $0.49 \pm 1.4$ , respectively). A similar gender pattern was observed in the deciduous dentition. With regards to oral hygiene, there were no statistically significant differences between the two genders ( $P=0.445$ ), and areas covered with plaque were  $24.7 \pm 12.0\%$  for boys and  $23.4 \pm 13.0\%$  for girls. Overall, 38% of children needed a referral to a dentist for operative dentistry procedures in either the permanent or deciduous dentitions. In addition, around 10% of examined children needed professional cleaning and 18% required fillings in their permanent dentition. There were no differences in dental treatment needs between children born in or outside of Canada ( $P=0.906$ ).

**Conclusions:** Overall, the oral health of inner-city elementary school-aged children is poor, their oral hygiene is unacceptable, and a considerable number need professional dental treatment.



**33. Characterization of Space Maintainers in Specialty Practice: Four-Year Retrospective Study**

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**Objectives:** Space maintainers are often utilized in pediatric dentistry and orthodontics to achieve space control. There are a number of different types, each with its own indication and purpose. Practitioner technique, patient compliance and appropriate usage all contribute to variability in the lifespan of space maintainers. The aim of this study was to characterize a sample of space maintainers inserted between 2000 and 2003 in one specialty pediatric and orthodontic private practice and determine if the intended lifespan was achieved.

**Methods:** Eligible subjects were identified through the practice lab requisition database. The records were located at three sites using information such as patient's legal name, date of birth and date of insertion. Data collection included: date of birth; gender; appliance type; dates of insertion; re-cementation and removal of the appliance; reasons for removal; type of cement used; oral hygiene; co-operation; and caries risk. De-identified data were recorded in a spreadsheet format for descriptive analysis.

**Results:** Over an eight-week period, 851 of 973 charts on the database list were able to be located (416 male, 435 female). The interim sample consisted of 439 lower lingual holding arches, 241 Nance and 171 band-and-loop appliances. Preliminary analysis indicated cement loss as the primary reason for failure. Inconsistency in oral hygiene, co-operation and caries risk measures between pediatric and orthodontic charts was apparent, leading to difficulties in achieving an unbiased sample.

**Conclusions:** Retrospective studies of this nature are labour-intensive and pose unique challenges such as chart retrieval, loss of subjects to follow-up and inter-clinician variability in technique and charting preferences. Despite these limitations, this retrospective study was able to characterize a large sample of space maintainers. A minority of the sample failed to survive for the intended lifespan. Detailed analysis of the final sample will provide invaluable insight into factors contributing to appliance failure.

**Acknowledgements:** Supported by a UBC Faculty of Dentistry Undergraduate Student Summer Research Award.

### 34. **Modulation of Elastase Activity of Cathepsin K**

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**Objectives:** Elastin degradation by proteases plays a critical role in the progression of cardiovascular, respiratory and skin diseases. Besides selected matrix-metallo and serine proteases, cathepsins K, S and V (L in mouse) represent the physiologically potent elastases. These cathepsins play an important role in bone metabolism and hormone and antigen processing. Earlier experiments showed the existence of two exosites in human cathepsin V whose modifications resulted in significant loss of elastase activity without affecting its enzymatic activity. Therefore, the present study was undertaken to identify the presence of similar exosites in cathepsin K that influence the elastase activity.

**Methods:** Cathepsin K exosite mutant was expressed in *Pichia pastoris* and purified using hydrophobic interaction chromatography. The enzymatic activities of the mutant, in addition to wild-type cathepsin K, were tested using a generic protease substrate gelatin as well as fluorometrically with the cathepsin K-specific synthetic substrate ZFR-MCA. The kinetic parameters such as  $K_m$ ,  $V_{max}$  and  $K_{cat}$  values for both of the enzymes were determined. The stability assays were carried out for the wild-type and the mutant protein at room temperature, 28°C and 37°C. The elastolytic activity of the mutant and the wild-type cathepsin K were determined using soluble and insoluble elastin substrates.

**Results:** Cathepsin K mutant protein demonstrated significantly similar kinetic values ( $K_m=2.1\mu M$ ,  $k_{cat}=3.8s^{-1}$ ) compared to the wild-type cathepsin K ( $K_m=3.0\mu M$ ,  $k_{cat}=4.2s^{-1}$ ). The proteins showed similar stability profiles. The enzyme concentration determined by active-site titration using E-64 was 234  $\mu M$ . However, the elastase activity of the mutant protein was reduced by 70-80% when compared to wild-type cathepsin K. Both proteins degraded gelatin at concentrations as low as 5 nM, demonstrating active-site functionality.

**Conclusions:** This study demonstrates the significance of exosites of enzymes as potential therapeutic targets to inhibit the degradation of selective and pharmaceutically highly relevant substrates with minimal side effects.

**Acknowledgements:** This study was supported by the Canadian Institutes of Health Research and a Canada Research Chair award to DB.

35. **Cycle Fatigue of Controlled Memory Wire Nickel–Titanium Rotary Instruments**

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**Objectives:** This study examined the fatigue behaviour of two types of nickel–titanium (NiTi) instruments made from a novel controlled memory NiTi wire (CM wire) under various environmental conditions.

**Methods:** Three conventional superelastic NiTi instruments of ProFile, Typhoon<sup>TM</sup> and DS-SS0250425NEY, and two new CM wire instruments of Typhoon<sup>TM</sup> CM and DS-SS0250425NEY CM were subjected to rotational bending at a curvature of 35° in air, deionized water, deionized water after immersion in 6% sodium hypochlorite for 25 min, or 17% ethylenediaminetetraacetic acid (EDTA), and the number of revolutions of fracture ( $N_f$ ) was recorded. The fracture surface of all fragments was examined by a scanning electron microscope. The crack-initiation sites and the percentage of dimpled areas compared to the whole fracture cross-section were noted.

**Results:** Two new CM wire instruments yielded an improvement of over four to six times in  $N_f$  compared to conventional NiTi files with the same design under various environments ( $P < 0.05$ ). The fatigue life of three conventional superelastic NiTi instruments was similar under various environments, while the  $N_f$  of two new CM wire instruments was significantly longer in liquid media than in air ( $P < 0.05$ ). The vast majority of CM instruments showed multiple crack origins, whereas most instruments made from conventional NiTi wire had one crack origin. The values of the area fraction occupied by the dimple region were significantly smaller on CM NiTi instruments than in conventional NiTi instruments under various environments ( $P < 0.05$ ).

**Conclusions:** Within the limitations of this study, the type of NiTi metal alloy (CM files versus conventional superelastic NiTi files) influences the cyclic fatigue resistance under various environments. The fatigue life of CM instruments is longer in liquid media than in air.

**Acknowledgements:** This research was supported in part by a Pilot Project Award from the UBC Faculty of Dentistry.

**36. Regular Dental Screening Facilitates Early Diagnosis of High-Risk Oral Lesions**

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**Introduction:** With ~60% five-year survival rates, oral cancer is often diagnosed at its later stages. Detection of lesions at earlier stages results in a better prognosis.

**Objectives:** (1) To characterize the experiences of patients with high-risk oral lesions (HRLs) from initial identification to diagnostic work-up. (2) To determine the factors that impact diagnostic time-delay according to patient experiences.

**Methods:** A survey-type questionnaire was used to collect data on patient experiences leading to an HRL diagnosis. Patients attending the BC Cancer Agency with HRLs and diagnosed within 12 months of the interview were invited to participate in this study.

**Results:** Among 150 patients interviewed, 91 (61%) patients were self-identified (SI) and 59 (39%) were identified by healthcare professional screening (PS; 88% by dental professionals). Significantly lower rates of invasive squamous cell carcinomas (SCCs) were identified by PS as compared with SI (46% vs. 77%,  $P=0.0001$ ). SI showed significantly higher rates of delay as compared with PS (58% vs. 36%,  $P=0.008$ ). The most common symptoms for SI were pain (77%) and non-healing ulcers (62%), which prompted these patients to seek healthcare attention, while the majority of patients (72%) in PS reported no symptoms. Dental professionals were the first healthcare professionals seen for significantly more pre-cancers (68% vs. 32%,  $P=0.0005$ ), while medical professionals were the first seen for significantly more late-stage SCCs (77% vs. 23%,  $P=0.0005$ ). Surprisingly, almost half (47%) of all patients indicated that they were not aware of the oral cancer.

**Conclusions:** Oral cancer screening by healthcare professionals, particularly by dental professionals, can play a critical role in the identification of earlier oral lesions at risk of cancer progression. Promotion of oral cancer awareness for both patients and healthcare professionals may facilitate earlier diagnostic work-up of oral lesions.

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**37. Connexins Regulate MMP-1 Expression and Function in Fibroblasts**

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**Objectives:** Connective-tissue fibroblasts communicate via cell–cell adhesions called gap junctions (GJ) that are composed of connexins (Cx). Interestingly, functional blockage of connexin43, the most abundant GJ protein expressed by fibroblasts, accelerates wound granulation tissue formation *in vivo*, but little is known about the mechanisms involved. Our goal was to determine the mechanisms by which connexins may promote wound healing. We hypothesized that Cx43 regulates the expression of MMPs, key modulators of inflammation, cell migration and tissue remodelling in fibroblasts.

**Methods:** Human gingival fibroblasts were cultured at high density allowing the cells to use connexin-mediated cell–cell communication. Expression of various connexins by fibroblasts was analyzed by real-time PCR, Western blotting and immunostaining. Cells were treated with peptides GAP27 or GAP26 that block Cx43 by different mechanisms, or with the chemical reagents carbenoxolone or meclofenamic acid, which non-specifically block connexin function. Expression of MMPs was then analyzed using real-time PCR and Western blotting.

**Results:** Fibroblasts expressed Cx43 as their major gap junction protein with moderate levels of Cx45 and very low levels of Cx40 and Cx32. At high cell density, Cx43 was functional as indicated by its localization at cell–cell contacts and distinct phosphorylation of its cytoplasmic tail compared to the low-density cultures. Treatment of cells at high density with all connexin inhibitors resulted in significantly increased expression of MMP-1. Furthermore, blocking of Cx43 resulted in increased secretion of active MMP-1 protein by fibroblasts.

**Conclusions:** Cell–cell communication mediated by Cx43 regulates expression of MMP-1, the major collagenase expressed by fibroblasts during wound healing.

**Acknowledgements:** Supported by the Canadian Institutes of Health Research.

**38. Resonance Frequency Analysis of Implants Placed in Grafted Bone**

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**Introduction:** Dental implants commonly replace missing teeth with a high degree of predictability and clinical success. One of the key factors in achieving this success is osseointegration, or direct attachment of the implant to surrounding bone. The stability of the implant can be measured using resonance frequency analysis (RFA).

**Objectives:** (1) To use RFA to determine if stability of implants placed in grafted bone is similar to that of implants placed in native bone and to compare RFA values for implants placed in sites treated with autogenous, allogenic or xenogenic bone grafts. (2) To determine if RFA values are affected by implant width and length.

**Methods:** RFA measurements were obtained at time of implant exposure. Stability values were compared with a history of bone grafting. RFA values were compared for implants grouped according to width and length.

**Results:** There were no statistically significant differences (SSD) among RFA measurements taken from buccal, lingual, mesial or distal sites. The RFA values did not differ ( $P=0.349$ ) among groups of implants placed in sites grafted with different lateral ridge augmentation materials (no augmentation  $78.2\pm 6.3$ ; autogenous  $81.0\pm 3.2$ ; allograft  $78.8\pm 5.5$ ; xenograft  $75.5\pm 6.1$ ). There were also no SSD ( $P=0.943$ ) among RFA values for implants placed in grafted or un-grafted sinuses, regardless of graft material used (no sinus lift  $78.2\pm 6.4$ ; allograft  $78.7\pm 4.9$ ; xenograft  $78.4\pm 5.1$ ). RFA values amongst implants with different lengths differed ( $P\leq 0.005$ ) unexpectedly, with the highest value in shorter implants 6-9mm( $82.7\pm 2$ ), and lower values for implants 10-12mm( $78.4\pm 6.1$ ) and  $\geq 13$ mm( $76.5\pm 6.3$ ). Implants of different widths showed SSD in RFA values ( $P<0.001$ ), but the main difference was between narrow implants of  $<3.5$ mm( $72.4\pm 5.6$ ) and regular diameter implants 4.0-4.3mm( $79.4\pm 4.7$ ) and wide diameter implants  $\geq 4.8$ mm( $80.2\pm 6.5$ ).

**Conclusions:** The stability of implants did not relate to either lateral augmentation or sinus material used. The difference in RFA values was associated with implant length and width.

**Acknowledgements:** UBC Periodontics residents for clinical data collection.

**39. Dental Care Access for Children with Special Care Needs**

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**Objectives:** Our research aims to: (1) identify challenges and enabling factors typically encountered by caregivers in securing appropriate and timely dental care for their children with special health care needs (CSHCN); (2) describe actual oral health status of CSHCN referred to the Department of Dentistry at BC Children's Hospital (DD-BCCH) and compare caregivers' perceptions of their child's oral health status; and (3) generate preliminary data regarding the CSHCN population at DD-BCCH as a basis for prospective research projects and advocacy.

**Methods:** This exploratory pilot study will include quantitative and qualitative components. A sample of CSHCN caregivers (n=65) will be interviewed either in person or by telephone using a pre-tested interview instrument. Closed-ended questions will include race/ethnicity, language spoken at home, caregiver education level, family structure, income range. Open-ended questions will be used to report caregiver's perception of their child's oral health status and enabling factors/barriers when accessing dental services. Demographic information, medical diagnosis, oral health status, chief dental complaint, referral date and source, dental benefit type, anticipated dental treatment summary will all be extracted from their child's dental record. Quantitative data will be analyzed using univariate and bivariate statistical tests. Qualitative data will be understood by thematic analysis.

**Results:** Recruitment and data collection is pending an approval certificate from UBC C&W BREB. The potential subject pool is approximately 500 caregivers over a 10-month recruitment period, with expectations of an excellent recruitment rate. Our experience suggests that caregivers are eager to share their stories.

**Conclusions:** Based on the characterization of oral health status of this sample, we may surmise that BC's CSHCN have unmet oral health care needs. Common themes in enabling factors and barriers experienced by caregivers attempting to access dental services for their child will also be identified. This information may be useful to support future advocacy for CSHCN.

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**40. Access to Dental Care: Perceptions of Affordability, Availability and Acceptability**

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**Objectives:** This research explored access to dental care in low-income communities from the perspectives of low-income people, dentists and related health and social service providers.

**Methods:** A case study was conducted in two rural communities in British Columbia. Semi-structured qualitative interviews were supplemented by descriptive population health and income statistics for the area and the low-income respondents. The analysis adapts Penchansky and Thomas's (1981) *Dimensions of Access Framework* to explore perceptions of need; evidence of unmet needs and three dimensions of access: affordability; availability; and acceptability.

**Results:** Sixty interviews were conducted with three cohorts: low-income adults (N=41); dentists (N=6); and health and social-service providers (N=13). Results describe the poor fit between private practice dentistry, public dental benefits and the oral health needs of low-income communities. Dentists and low-income patients alike explained how the current model of private dental practice, fee-for-service payments and public dental benefit plans do not work well for either party. The major barriers for dentists and low-income communities seem to be the financial demands of dentistry and the cultural conflicts that occur when people from low-income communities attend private dental practices.

**Conclusions:** The research raises questions about the capacity of private practice dentistry to meet the public oral health needs of economically vulnerable groups. Improving access requires policies that address poverty and the enhancement of public dental benefits; however, addressing these financial barriers will not ensure access. Addressing the non-financial vulnerabilities would mean broadening the treatment options beyond the private practice business model to alternatives including community dental clinics sensitive to the unique needs of low-income populations.

**Acknowledgements:** This research was supported by a grant from the Canadian Institutes of Health Research (NET Grant #87104).



#### 41. **Effect of Detergents on the Antibacterial Activity of Disinfecting Solutions**

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**Objectives:** Detergents have been included in different disinfecting solutions to enhance their antibacterial effects. The purpose of this study was to evaluate the effectiveness of dentin disinfection by different antibacterial solutions in the presence and absence of detergents using a novel dentin infection model and confocal laser scanning microscopy (CLSM).

**Methods:** Semi-cylindrical dentin specimens were prepared from single-rooted teeth and infected with *Enterococcus faecalis* according to a previously described protocol by centrifugation. After 1 d incubation, the outer sides of the infected dentin samples were closed by nail varnish and the specimens were subjected to 1 min and 3 min exposure to sterile water, 0.1% Cetrimide (CTR), 2% sodium hypochlorite (NaOCl), 6% NaOCl, Chlor-extra (Vista Dental, Racine, WI), 2% chlorhexidine (CHX), CHX-plus (Vista Dental, Racine, WI), Iodine, or Iodine+0.1% CTR. Specimens were then washed in sterile water for 1 min and vertically fractured through the root canal into two halves for viability staining and CLSM examination to analyze the proportions of dead and live bacteria inside dentinal tubules.

**Results:** More bacteria in the infected dentinal tubules were killed after 3 min exposure than after 1 min exposure to the disinfecting solutions in all experimental groups ( $p < 0.05$ ). The antibacterial solutions with detergents (CTR, Chlor-extra, CHX-plus, Iodine+0.1% CTR) showed a higher proportion of dead bacteria than the corresponding solutions without detergents (sterile water, NaOCl, CHX, Iodine) ( $p < 0.05$ ). Six percent NaOCl and Chlor-extra were the most effective solutions in killing bacteria, both resulting in over 45% and 65% dead bacteria after 1 min and 3 min exposures, respectively. Only 3-4% of the bacteria were dead in the sterile water group, while 0.1% CTR alone was able to kill 24-36% of the bacterial cells.

**Conclusions:** Addition of detergents to the disinfecting solutions increased their antibacterial effect against bacteria in dentinal tubules.

## 42. **The Effect of Peptidase Inhibitor 15 on Skeletal Development**

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**Objectives:** Peptidase inhibitor 15 (PI15) was first identified from cultured medium of human glioblastoma cells as a novel 25-kDa protein (P25TI) with weak inhibitory activity to trypsin. The main role of PI15 is not clear, but the misexpression of the PI15 gene in the chicken embryo face inhibited bone formation in the upper face and induced extra digits in the limbs. The aim of this study was to determine whether PI15 has direct effects on the cells that make cartilage or bone.

**Methods:** Mesenchymal stem cells from the mandible, maxilla and the hind limbs were isolated from stage 24 chicken embryos. The source of PI15 was the media collected from a DF-1 chicken fibroblast cell line engineered to secrete human PI15-EGFP fusion protein. Cells were plated in high-density micromass cultures. Conditioned media was added 24 hours after initial attachment of the cultures and was diluted 50:50 with DMEM-F12 media. Media was changed every second day. The cultures were grown for 2-8 days and stained for cartilage and bone using Alcian blue staining and alkaline phosphatase staining, respectively.

**Results:** Normally in mandibular cultures, many nodules of cartilage form. However, PI15 acts during early condensation formation to directly inhibit cartilage formation. The overall size of the cultures was unchanged. When the hind limb cultures were treated with PI15, the area of cartilage formed was increased and again culture size was unaffected. Maxillary cultures did not form any cartilage and PI15 did not change this phenotype.

**Conclusions:** PI15 is sufficient to disrupt chondrogenesis in a spatially restricted manner. Future studies are required to determine the reasons why the effects of PI15 are different in cells from different regions of the embryo.

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