Abstract #1: Beliefs of Lay People Concerning Periodontal and Cardiovascular Disease Association

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Objectives: To identify beliefs concerning the potential links between periodontal and cardiovascular diseases held by members of the general public attending a community health clinic.

Methods: A literature review was conducted utilizing MEDLINE and OVID. Keywords used included cardiovascular disease (CVD), coronary artery disease, bacterial endocarditis, oral health, periodontitis, and gum disease. Fifty-three articles were chosen based on their abstracts, read fully and, from the reference lists of these articles, a further seven articles were found. An 11-item questionnaire was developed and distributed at the Mid-Main Community Health Clinic in Vancouver, Canada. Fifty-three completed surveys were collected. The questionnaire explored what the general public knew about the links between periodontal and cardiovascular diseases.

Results: There are many reports and research efforts that show the potential links between periodontal and cardiovascular diseases, but no research has been done surrounding the beliefs held by the general population in this regard. Fifty-three percent of survey responses came from females. The mean age of the entire sample was 41 years old. Forty-seven percent of females and 35% of males believed that oral health had no or a low risk for the development of cardiovascular problems. Although 65% of males rated their oral health as excellent or very good, 80% of them had bleeding gums while brushing or flossing. The percentages for females were 71 and 57, respectively. Among all participants who said there is a moderate/high risk of developing CVD through oral disease, 10% of them rated their oral health as poor.

Conclusions: Although the literature has discussed potential links between these two diseases extensively, a considerable portion of survey respondents seemed to be unaware of such links. There is a possible gap between scientific research findings and lay public belief systems.

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Abstract #2: Ethnic Differences in the Survival of Oral Cavity and Oropharyngeal Cancers

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Objectives: We have previously reported ethnic differences in the incidence of oral cavity cancers (OCC) and oropharyngeal cancers (OPC) in British Columbia (BC). Variations in mortality and survival rates have not previously been ascertained. In the present study, we determined tumour stage at diagnosis, 2- and 5-year disease-specific survival rates, and age-adjusted mortality rates for South Asians (SA) and the general population (GP) of BC. These data are presented by gender.

Methods: All OCC and OPC data were retrieved from the BC cancer registry database and ethnicity was determined using surname lists. Descriptive statistics, Kaplan Meier curves, life table actuarial methods, and the Cox Proportional Hazards Model were used to calculate and compare survival rates.

Results: Late-stage diagnoses for OCC were more prevalent in the GP at 47% in males and 40% in females. For OPC, in men it was more for the GP at 82% and for women it was more in SA at 83%. For OCC, SA had lower 2- and 5-year survival rates in both genders; in men, rates were 70.1 and 60.9, respectively, and in women, rates were 68.5 and 60.5, respectively. For OPC, the GP had lower 2- and 5-year survival rates; in men, rates were 70.5 and 59.0, respectively, and in women, rates were 59.4 and 59.2, respectively. Age-adjusted mortality rates were higher for SA men and women: 1.4 and 2.2 times of the GP.

Conclusions: SA had higher disease-specific age-adjusted mortality rates and poorer survival rates for OCC in both genders. There is a need for targeted and culturally appropriate prevention and screening programs, especially among SA.

Acknowledgements: AA is supported by a Psychosocial Oncology Research Training Fellowship from the Canadian Institutes of Health Research.
Abstract #3: Proteomic Identification of Substrates Cleaved by MMP-12 in Arthritis

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Objectives: Matrix metalloproteinase-12 (MMP-12), a macrophage-specific endopeptidase, precisely cleaves neutrophil-recruiting ELR⁺-CXC chemokines and monocyte chemotactic proteins CCLs thereby regulating the influx of inflammatory cells. Here we hypothesized that MMP-12 processes additional signalling molecules involved in promoting or resolving inflammation.

Methods: To identify new MMP-12 substrates we applied the novel proteomic technique termed Terminal Amino Isotopic Labelling of Substrates (TAILS). By this method the neo-N-termini generated by the protease of interest as well as all other N-termini in a proteome sample are isotopically labelled at the free amino groups by iTRAQ-reagent. Following trypsin-digest, sample complexity is reduced by selective removal of internal unlabelled trypsin-generated peptides by a polyaldehyde-polymer, thereby enriching the labelled original and neo-N-terminal peptides. These are then identified by tandem mass spectrometry. Differential labelling of samples containing active or inactive protease allows the quantitative comparison of peptides from substrates and non-cleaved proteins present in the different samples. By a new rigid bioinformatics pipeline (CLIPPER), cleaved proteins are identified with high confidence and thereby distinguished from the natural N-terminome and basal proteolytic products.

Results: We analyzed secreted proteins in the (1) secretome of Mmp12⁻/⁻ fibroblasts incubated with recombinant MMP-12 or buffer only; (2) secretome of unstimulated or TNF-α stimulated macrophages (RAW 264.7 cells) incubated with recombinant MMP-12 or buffer only; (3) peritoneal lavages of thioglycolate-injected Mmp12 wild-type and knockout mice; and (4) arthritic paws of Mmp12 wild-type and knockout mice.

Conclusions: In each analysis ~1,000 proteins were identified with high confidence, of which ~30 were high confidence substrates in the in vivo models of inflammation. Among the novel MMP-12 substrates are cytokines (EMAP-II), growth factors (IGFBP-3), extracellular matrix components (sulfated glycoprotein-1), or pro-inflammatory molecules such as granulins. The richness of the data sets provides many new starting points for ongoing studies on the biological roles of MMP-12 in inflammation.
Abstract #4: Pharmacological Inhibition of Cathepsin S Decreases Atherosclerotic Plaque Size and Vulnerability

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Objectives: Recent studies provided evidence for a significant role of cathepsin S during extracellular remodeling in atherosclerosis. In this study, we investigated the effect of a specific cathepsin S inhibitor on atherosclerotic plaque progression in the brachiocephalic artery.

Methods: Male and female ApoE-/− mice on a cholate-containing high fat diet containing or lacking a specific cathepsin S inhibitor were evaluated for the remodeling of atherosclerotic lesions. After 8 weeks of the diet, brachiocephalic arteries were analysed for plaque size, collagen, macrophage and smooth muscle cell content, for elastic lamina breaks, and for the number of buried fibrous caps.

Results: The size of atherosclerotic plaques was significantly reduced in inhibitor-treated mice and they also showed a significantly smaller number of elastin laminae breaks, as well as a lower number of plaque macrophages and buried fibrous caps. The in vivo efficacy and specificity of the cathepsin S inhibitor was revealed by the accumulation of the p10 fragment of the invariant chain in the spleen.

Conclusions: The selective inhibition of cathepsin S shows a strong atheroprotective activity, demonstrating the potential benefits of small molecule anti-cathepsin therapy.

Acknowledgements: This study was supported by a research grant from the National Institutes of Health.
Abstract #5: Oral Health Promotion in the Community: The PACS Module

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Objectives: To promote oral health in two inner city elementary schools in Vancouver as part of the Community Service Learning through the PACS module.

Methods: Situational and audience analysis, literature review, and oral health promotion planning in collaboration with the schools and community site. Satisfaction survey questionnaire development. Evaluation and sustainability reports.

Results: Two oral health promotion projects were implemented with the assistance of the Mid-Main Community Health Centre. In one project, school visits to David Livingston and General Brock Elementary Schools were made and educational presentations were successfully performed for three separate classes ranging from Grades 2-4. The presentation at General Brock Elementary School was filmed, highlighting the short play and the interactive small group sessions aimed at teaching proper brushing and flossing techniques and identifying cariogenic food. Prior to the school visits, questionnaires were distributed to the parents and their scores were compared to those obtained by the students at the end of the school visits. The second project involved patient satisfaction assessment via a written survey at the Mid-Main Dental Clinic.

Conclusions: Inner city school children were provided with the answer key to the quizzes, pamphlets for their parents, as well as samples of toothbrushes, toothpaste, and floss. These take-home packages provided a means for parental involvement and extended the education beyond the limited presentation time. The survey had a relatively low response rate with 58 surveys completed over three and a half weeks. The clinic received high approval ratings on all categories assessed, ranging from friendliness to technical knowledge and expertise.

Acknowledgements: Mid-Main Community Clinic, the PACS instructors, and the UBC Faculty of Dentistry Dean’s Office for supporting the project and the posters.
Abstract #6: Social Networking Sites for Promoting a Dental Health Education Website

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Filipino immigrants in Metro Vancouver have suggested the Internet as a key platform for delivering culturally appropriate dental health information in various digital formats. An Ontario study revealed that among Filipino immigrants, the Internet is the primary means of communication and information exchange between family and friends in and out of Canada. In 2008, a global survey revealed that social networking was the most common activity among Filipino Internet users. Additionally, Filipinos were singled out as the largest group and most active users on Friendster and Multiply.

Objectives: To assess the feasibility of using Internet-based social networking sites to promote a dental health education website.

Methods: A dental education website (Pinoy Smile www.pinoysmiles.net) was developed and hosted on blogspot.com, a popular social networking site (SNS). The target demographic of Filipino parents and childcare providers was identified through searching Friendster, Multiply, Blogspot, and Facebook using keywords. A comment or message with a link to Pinoy Smile was left on the sites of 100 randomly-selected SNS users. Using Google Analytics™ and Sitemeter™, website traffic was tracked for 45 days.

Results: Pinoy Smile had 8,608 visitors from 66 countries and territories making a total of 11,762 visits during its first 45 days of Internet broadcast. Top source countries were the Philippines (8,179), Canada (1,700 visits), United States (561), Hong Kong (287), and United Arab Emirates (234). The most popular section was the “Smile of the Month” contest, garnering 10,891 of the site’s total 27,825 page views. Direct traffic accounted for the majority of visits (59.4%), SNS referrals for 39%, and search engines for 1.6%. Top referrers were Facebook (21%), direct e-mail (12%), and the Pinoy Smile share widget (8%).

Conclusions: Results suggest that for the Filipino community, social networking sites are useful in promoting and increasing traffic to a new dental health education website.

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Abstract #7: Unique FISH Patterns Associated With Poor Outcomes of Oral Lesions

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Subgroups of oral leukoplakia are at an extremely high risk for cancer development despite complete excision. Histology alone is a relatively poor indicator.

Objectives: The goal of this study was to identify unique genetic alterations that may facilitate the identification of oral lesions with high-risk behaviour.

Methods: The study involved a unique tissue array comprised of 54 1.0-mm tissue cores of normal (19 cases) and premalignant lesions (35 oral dysplasias). Twenty of the dysplasias were refractory to treatment (D-RTT) with repeated recurrence or progression after excision. The remaining dysplasias were randomly chosen from the BC Oral Biopsy Service to represent the general dysplasias population (D-GP). Three dual colour probe sets were used: EGFR(7p12)/CEP7, CyclinD1(11q13)/CEP11, and p16(9p21)/CEP9. Samples with adequate DNA (19/20 D-RTT and 8/15 D-GP) were analyzed for molecular risk using microsatellite analysis at 3p14, 9p21, and 17p.

Results: Although there was no significant difference in demographics and degrees of dysplasia between D-RTT and D-GP, the two groups displayed different LOH molecular risk patterns: 17 (89%) of the D-RTT showed loss of 3p and/or 9p, and 11 (58%) showed additional loss at 17p. These loss patterns were not observed in the D-GP (P<0.0001). High genomic gain in EGFR and Cyclin D1 signals was identified in 8/20 (40%) and 14/20 (70%) of the D-RTT and only 1 (7%) and 2 (13%) for D-GP (P=0.0002 and <0.0001, respectively). The D-RTT group also showed a significant increase in both CEP7 and CEP11 trisomy and polysomy. In contrast, alterations at CEP9 are less common.

Conclusions: The data support the potential use of FISH profiles to identify clinically aggressive premalignant lesions, especially when such lesions are present as small clones in tissue samples. Such markers might be used to identify lesions requiring more aggressive treatment and/or novel molecular targeted chemoprevention.
Abstract #8: Three-Dimensional Soft Palate Modeling from Magnetic Resonance Imaging Data

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Objectives: This study was designed to build a computational three-dimensional (3D) model of the soft palate biomechanics from a Magnetic Resonance Imaging (MRI) data set.

Methods: Multiple MRI slices of the head and neck region of a young non-overweight Caucasian male volunteer were taken in the supine position with a passive oral appliance (OA) in place. The DICOM MRI slices were transcoded into Analyze7.5 format and registered into a high-resolution volumetric data set using the Amira® software package. Amira® software is capable of performing a computer-assisted tracing of the soft palate contour based on anatomical structure and tissue contrast of the images. The contour traced on one direction was projected on the other two directions, allowing for modifications to be made if contours did not agree with each other. A surface mesh was generated from the final segmentation and used to create a volumetric tetrahedral mesh needed for finite element simulation with the ArtiSynth 3D modeling platform. In order to simulate the individual muscle movement of the soft palate, multiple landmarks of each muscle was group selected in Amira®.

Results: The segmented soft palate complex consisted of five groups of muscles: levator veli palatini, tensor veli palatini, palatoglossus, palatopharyngeus, and musculus uvulae. The palatine tonsil, which is located between the pharyngopatine and glossoopalatine arches, was inevitably included in the segmentation. The levator and tensor veli palatinis were less identifiable due to the overlapping soft tissue structures in the nasopharyngeal airway area.

Conclusions: Both the volume mesh and landmarks were integrated with an existing 3D biomechanical model of a human jaw and larynx in ArtiSynth to provide a virtual representation of the oropharyngeal region for diagnostic, treatment planning, research, and teaching purposes. We continue to investigate how to simplify the time-consuming segmentation process without compromising the quality of the segmentation.
Abstract #9: Comparative Effectiveness of Listerine, Chlorhexidine and Modified Chlorhexidine Against Bacteria

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Objectives: Having an effective irrigating solution is essential for the success of endodontic treatment. An irrigating solution with bactericidal activity is critical for eradication of bacteria that reside in the necrotic root canal and that cause infections in the oral cavity. In the present study, we investigated the bactericidal effects of various existing and novel mixtures of medicaments on planktonic Enterococcus faecalis and suspended mature plaque.

Methods: Listerine, Chlorhexidine, and new modifications of Chlorhexidine were incubated for different experimental times with E. faecalis cells suspended in sterile water. A sample of the mixture was then taken and serial ten-fold dilutions were made and plated on tryptic soy agar plates. The plates were examined for growth after 24 hours of incubation at 37°C. A suspension of plaque bacteria from the oral cavity was also exposed for different lengths of times to the same medicaments. The samples, after serial dilutions, were inoculated on rich, non-selective blood agar plates. Examination for growth was done after 48 and 72 hours of incubation at 37°C under anaerobic conditions.

Results: Listerine killed all E. faecalis cells and plaque bacteria in 10 seconds. Chlorhexidine at 0.12% required over 10 minutes to completely eradicate E. faecalis. The modified, new mixtures of Chlorhexidine had the same bactericidal effect as Listerine against E. faecalis and plaque bacteria.

Conclusions: The novel mixtures of 0.12% Chlorhexidine were equally effective in instant killing of bacteria as Listerine. Future studies will show whether the excellent long-term effects of Chlorhexidine are still present in the modified/new Chlorhexidine mixtures. The combination of effective instant killing and long-term effects could potentially give these new mixtures a significant role in the prevention and eradication of oral infections.

Acknowledgements: This study was supported by funding from the Faculty of Dentistry at the University of British Columbia and a UBC Faculty of Dentistry Undergraduate Summer Research Student Award.
Abstract #10: Role of GSK-3β in Epithelial Mesenchymal Transition of Murine Palatal Fusion

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Objectives: Glycogen synthase kinase 3β (GSK-3β) is an important kinase in the regulation of Epithelial Mesenchymal Transformation (EMT). Previous studies have shown that in malignant tumour cells, GSK-3β regulates EMT through the transcription factor SNAIL, which downregulates E-Cadherin expression and promotes mesenchymal confluence. We hypothesized that this mechanism also occurs during murine palatal fusion. Our hypothesis was supported by recent studies stating that GSK-3β conditional knockout mice develop a secondary palatal cleft. We believe that GSK-3β is being mediated by the TGF-β3 pathway during palatal fusion. The results will support the notion that EMT is the major mechanism leading to palatogenesis.

Methods: In vivo – The embryonic heads (wild-type and TGF-β3−/−) at E14.25 were fixed in 4% paraformaldehyde-PBS and embedded in paraffin. Immunohistochemistry was performed with antibodies against Snail, E-cadherin, GSK-3β, and p-GSK-3β. In vitro – Palatal shelves were dissected at E13 and cultured for 24h with 50 ng/ml of recombinant human TGF-β3 (rhTGF-β3) and processed for immunohistochemistry. Sections were stained with antibodies against as well as in vivo. GSK-3β and p-GSK-3β levels were examined by Western blot analysis in the palatal midline following treatment with rhTGF-β3.

Results: In vivo – During critical stages of palatogenesis associated with palatal shelf adherence and midline seam breakdown, GSK-3β and p-GSK-3β were expressed at high levels in WT samples, specifically in the MEE. Snail translocated into the nucleus in the cells in which p-GSK-3β was strongly expressed. P-GSK-3β expression within the MEE was reduced in TGF-β3−/− samples and Snail remained in the cytoplasm. In vitro – The exogenous rhTGF-β3 was able to induce p-GSK-3β expression in the MEE in rhTGF-β3-treated organ culture samples.

Conclusions: During palatal fusion in the MEE, (1) GSK-3β signalling is activated by TGF-β3, and (2) p-GSK-3β regulates Snail, which then translocates into the nucleus and downregulates E-cadherin expression, promoting EMT and palatal fusion.

Acknowledgements: This project was supported by a grant from the National Institute of Dental and Craniofacial Research, R01 DE16296.
Abstract #11: Application of a Novel Wound Healing Model in Mouse Skin

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Objectives: Many transgenic and knockout mouse lines currently available can provide unique tools for the study of molecular mechanisms involved in skin wound healing and scar formation. However, in contrast to human skin, wound closure in mouse skin occurs fast and mostly by contraction, rarely resulting in any type of scar formation. Therefore, we investigated the utility of a novel mouse skin wound healing model to more closely mimic human skin wound healing. We hypothesized that the splinting of experimental mouse skin wounds would reduce wound contraction, resulting in slower wound healing.

Methods: A full-thickness, excisional skin wound was created using a 4 mm biopsy punch in each side of the upper back of mice (n=22 mice). A standard silicone splint was attached around the first wound while the other wound was left to heal non-splinted. Wound size was measured from standardized digital images over time. Wound closure and molecular composition were studied from histological sections obtained from wound biopsies at days 2, 4, and 7 after wounding by histomorphometry and immunostaining.

Results: Wound sizes remained relatively constant until day 4 post-wounding, after which the wound contraction proceeded faster. By day 7, the non-splinted wounds were strongly contracted and showed a smaller surface area compared to the splinted wounds. This difference was not statistically significant due to the loss of a number of splints over time. Wound closure and re-epithelialization occurred, however, more slowly in the splinted than in the non-splinted wounds (closed at days 7 and 4, respectively). Accumulation of tenascin-C was delayed in splinted wounds as compared to non-splinted wounds, indicating delayed granulation tissue formation.

Conclusions: Splinting of experimental mouse skin wounds may reduce wound contraction and delay wound closure, more closely mimicking human skin wound healing. Better methods for anchoring the splint are needed to improve the reproducibility of the method.

Acknowledgements: Supported by grants from the Canadian Institutes of Health Research including a CIHR Health Professional Student Research Award.
Abstract #12: Expression of Interleukin-1α in Kindlin-1 Deficient Keratinocytes

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Objectives: Kindler syndrome is an autosomal recessive disorder characterized by loss of the kindlin-1 protein, resulting in blistering of the skin and colon mucosa, and development of early-onset aggressive periodontitis. Kindlin-1 is a focal adhesion protein involved in integrin activation. Failure to activate integrins may result in reduced epithelial adhesion in the skin and between the tooth and gingival tissue, leading to blistering, but the exact mechanism remains unclear. We hypothesized that kindlin-1 regulates pro-inflammatory cytokine expression, particularly IL-1α, which leads to inflammatory changes that result in blistering.

Methods: Expression of kindlin-1 was knocked down in cultured HaCat keratinocytes using short interfering RNA (siRNA). Expression of kindlin-1 mRNA and protein levels was assessed by real-time RT-PCR and Western blotting, respectively. Expression of IL-1α, IL-1R1 (IL-1 receptor 1), and IL-1RA (IL-1 receptor antagonist) mRNA was measured by real-time RT-PCR. IL-1α and type VII collagen protein levels in cell culture medium were measured using ELISA.

Results: Expression of kindlin-1 mRNA and protein levels in HaCat keratinocytes was knocked down by 85-90% using kindlin-1 siRNA as compared to cells treated with control siRNA. In four independent experiments, expression of IL-1α mRNA was increased by approximately three-fold in kindlin-1 knockdown keratinocytes as compared to control cells. IL-1α protein levels were correspondingly increased in the conditioned medium of the knockdown keratinocytes. Conversely, mRNA expression levels of IL-1R1 or IL-1RA remained unaltered. In accordance with elevated type VII collagen expression in Kindler syndrome patients, expression of type VII collagen was found to dose-dependently increase in IL-1α-treated skin fibroblasts.

Conclusions: The results indicate that kindlin-1 regulates IL-1α expression and secretion in cultured keratinocytes. Therefore, increased IL-1α expression may participate in the pathogenesis of blister formation in Kindler syndrome. IL-1α may also contribute to the disturbed type VII collagen deposition, a characteristic histological feature of this disorder.

Acknowledgements: This study was supported by a grant from the National Institutes of Health.
Abstract #13: Factors Influencing Dentists’ Decisions to Treat Patients in Long-Term Care

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Objectives: The purpose of this study was (a) to determine factors that influence dentists in their decision to treat in long-term care (LTC) facilities within BC; (b) to determine if dentists practicing in rural areas of BC are more willing to provide services in LTC compared to dentists in urban areas; and (c) to assess if there were any changes in opinions in treating patients in LTC since 1985.

Methods: A package containing 3 forms for dentists who currently treat, have never treated, or who have stopped treating patients in LTC was mailed out to 800 dentists throughout BC. Dentists filled out one of the surveys and faxed in back to the BCDA. A reminder to fill out the survey was sent by fax and e-mail after 2.5 weeks.

Results: A response rate of 31.4% was achieved in this study. Seventy-five percent of the responding dentists who currently treat patients in LTC reside outside of Metro Vancouver, 78% of dentists who had stopped treating patients in LTC reside outside of Metro Vancouver, and 58.2% of dentists who had never treated patients in LTC reside outside of Metro Vancouver. Professional and personal factors were the main determinants in the provision of treatment.

Conclusions: Analysis identified that dentists who treat patients in LTC felt that it was a part of their professional responsibility to treat institutionalized elders. The lack of a dental operatory and lack of experience/training in geriatric dentistry were the main concerns of dentists who never provided services. Compared to 1985, dentists in 2008 showed increased awareness of the need for dental services by patients in LTC facilities. Dentists in rural areas were more involved with providing services to patients in LTC compared to their urban counterparts. Dentists who never provided services in LTC expressed interest in providing services in LTC.

Acknowledgements: This study was supported financially by the British Columbia Dental Association.
Abstract #14: Short-Term Clinical Outcomes of Nobel Active Implants: A Retrospective Multi-Centre Analysis

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The new Nobel Active implant system, pre-launched in 2007, was designed with numerous advantageous design properties including platform switching, self-drilling, bone condensing, and redirecting capabilities. It is proposed that these key design features confer higher insertion torque in soft bone and extraction sockets, thereby providing greater primary stabilization.

Objectives: A retrospective review of charts was conducted to assess the survival rate and marginal bone loss around Nobel Active implants after one year of loading.

Methods: The implants were placed by experienced practitioners in two private clinics and by senior Graduate Periodontics residents at the University of British Columbia (UBC). A total of 145 Nobel Active fixtures were evaluated from 84 patients. Of the assessed implants, 52 were placed immediately into extraction sockets and 93 into healed sites. The marginal bone loss was evaluated in conventional and digital periapical radiographs. Conventional radiographs were photographed and measurements were made using Image J 1.42 software (National Institutes of Health, USA) and Planmeca Romexis 2.2.7R software (UBC). Each radiograph was calibrated using the known implant length. The coronal aspect of the fixture was used as a reference point for measurements and the true bone resorption (the distance from the initial bone level to the bone level at follow-up examinations) was calculated.

Results: Six (4.1%) of the 145 implants failed and were removed during the follow-up. One of the failures occurred in a smoker, two in ex-smokers, and one in a diabetic patient. Further post-operative data will be collected in the Spring of 2010 and the results for marginal bone loss will be processed at that time.

Conclusions: Preliminary data indicates that clinical outcomes and failure rates of the Nobel Active implant system are comparable to that of current validated implant systems.
Abstract #15: Oral Health, Body Image and Social Interactions Amongst Institutionalized Elders

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Objectives: A positive body image is linked to increased social contacts as well as improved health, well-being, and quality of life, irrespective of age. However, the extent to which oral health and diseases influence body image and social interactions amongst frail elders in care facilities is unknown. A preliminary study of these relationships suggested that oral health may have an important influence on the body image and social interactions of institutionalized elder women. The current study has expanded this investigation to explore these relationships within a more diverse group of frail elders. The research question underlying this study is “how are the social interactions and body image of institutionalized frail elders influenced by perceived oral health and disease?”

Methods: Open-ended interviews were conducted with a purposefully selected group of cognitively intact, institutionalized elder men and women who exhibited varying degrees of frailty, social engagement, and oral health conditions. The narratives were analyzed using a constant comparative technique, and second interviews with the participants were conducted to check trustworthiness of the analysis.

Results: Five major themes emerged from the analysis: (1) institutional culture; (2) frailty and social context; (3) cleanliness; (4) priorities; and (5) perceived access to care.

Conclusions: Perceived oral health and body image among institutionalized elders are influenced by comfort, hygiene, and function. These findings are similar to that of elders who reside in the community; however, the degree to which social interactions are negatively impacted seems to be decreased and dependent on increasing frailty, institutional culture, and how an elder prioritizes their care.
Abstract #16: Structural Requirements for the Collagenase and Elastase Activities of Cathepsins K and V

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Objectives: Human cathepsins K, V, and L are proteolytically potent and structurally very similar cysteine proteases. However, their activities towards extracellular matrix proteins such as triple helical collagens and elastin are dramatically different. Human cathepsin K is a highly effective collagenase and cathepsin V is the most potent elastase among mammalian proteases. In contrast, human cathepsin L lacks any significant collagenase or elastase activities. The mechanism of their activities, however, is unknown.

Methods: Site-directed mutagenesis was used to characterize the role of cathepsin K specific residues in collagen degradation. The denaturation of collagen was determined by circular dichroism, and structural information of the complexes was gained by atomic force microscopy. To investigate the structural determinants of the elastase activity of cathepsin V, several chimera mutants between cathepsin L and cathepsin V were constructed. The mutant proteins were expressed, purified, and their elastolytic activities were determined.

Results: In this study, we determined that collagenolytically active cathepsin K requires the formation of a specific oligomeric complex between cathepsin K molecules and glycosaminoglycan chains. The complex forms a central pore which facilitates the partial unfolding of triple helical collagen prior to their cleavage. Cathepsins V and L share an amino acid sequence identity of about 80% but only cathepsin V exhibits a potent elastase activity. We demonstrated that two distinct exosites in cathepsin V facilitate its elastase activity. Site-directed mutagenesis analysis identified two small surface areas on cathepsin V which are remote from the catalytic site and the traditional substrate subsite binding sites. Each of the exosites contributes to the elastase activity of the protease in roughly similar proportions and may act to bind elastin.

Conclusions: Complex formation and the presence of exosites explain the unique specificities of cathepsins K and V towards biologically relevant and proteolytically rather resistant substrates.

Acknowledgements: This study was supported by a research grant from the National Institutes of Health.
Abstract #17: Misexpression of the RA Inactivating Enzyme CYP26A1 Inhibits Jaw Development

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Objectives: Our aim was to study the effects of reducing retinoic acid (RA) levels in the embryonic face on jaw morphogenesis. To achieve this we have targeted the final steps in the RA metabolic pathway, the breakdown of retinol products. The cytochrome P26 class of enzymes is responsible for retinoic acid breakdown in tissues. Therefore we increased the levels of one of these enzymes, CYP26A1, in the facial prominences. We hypothesized that lowering RA levels would either affect jaw patterning and/or cytodifferentiation.

Methods: Human CYP26A1 was cloned into the avian retrovirus, RCAS. Embryos at HH-15 (somite stages 23 to 28) were injected with RCAS::hCYP26A1 medial to the nasal pit, maxillary region, and mandibular arch using a glass needle. Embryos were fixed 12 days later at HH38 to examine external as well as skeletal anatomy. Expression of the virus and RA target genes was analyzed with in situ hybridization.

Results: When RCAS::hCYP26A1 was injected into the frontonasal mass or maxillary prominences, unilateral clefts formed on the treated side (7/20). Occasionally there were bilateral clefts. There were more cases of bilateral clefts in Mx injections than in FNM injections (4/17), possibly due to injection technique. Mandibular injections resulted in dramatic reductions in the length of the lower beak and occasional deviations. The external appearance is consistent with an inhibition of outgrowth of the facial prominences at an earlier stage which has led to a loss of skeletal elements. It is very likely that more subtle phenotypes will become apparent in the analysis of cleared skulls.

Conclusions: Increased expression of CYP26A1 appears to be an effective way to decrease RA levels. There does not seem to be any spatial restriction of the effects of CYP26A1, therefore RA is required for outgrowth of all facial prominences.

Acknowledgements: The authors thank Cheryl Whiting for cloning the CYP26A1 virus. This study was supported by Canadian Institutes of Health Research grants to JMR.
Abstract #18: The Role of Wnt5a in Mandibular Chondrogenesis

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Objectives: Previous studies from our laboratory have shown that Wnt5a, a Wingless-related protein, is expressed at high levels in the mesenchyme of all the facial prominences. Our aim was to use high density micromass cultures to study the effects of Wnt5a conditioned media on differentiation of bone and cartilage. Since mice with a targeted deletion of Wnt5a have smaller mandibles, we hypothesized that adding Wnt5a would increase bone formation.

Methods: Micromass cultures containing mandibular mesenchymal tissue from stage 20-24 chicken embryos were incubated for 8 days in a mixture of conditioned media from cells expressing Wnt5a or empty vector and complete media (DMEM:F12, 10% FCS). Adobe Photoshop was used to quantify the area of bone, cartilage, and the culture. One-way ANOVA followed by Tukey’s postoc testing (Statistica) was used to determine differences in matrix and culture size. Cell proliferation was assessed at 4 days by counting BrdU-labelled cells and PNA was used to stain cartilage condensations.

Results: Wnt5a-treated cultures were the same size as control cultures but had almost total loss of cartilage after the 8-day incubation period. Intriguingly, Wnt5a had very little effect on intramembranous bone formation; thus the effects were mainly on pre-chondrogenic cells. Cell proliferation was similar in experimental and control cultures; therefore the loss of cartilage progenitor cells was not due to an overall decrease in proliferation. Surprisingly, cartilage condensations formed as normal in the early cultures but did not differentiate. Further analysis of the canonical (via beta Catenin) and non-canonical (via JNK, Ror2) signalling pathways affected by Wnt5a will be presented.

Conclusions: Wnt5a has specific inhibitory effects on cartilage differentiation that occur after the condensation stage. Our data show it is important to regulate the level of Wnt5a signalling during normal development and that excessive Wnt5a activity is predicted to prevent formation of the chondrocranium.

Acknowledgements: This project was funded by CIHR grants to JMR.
Abstract #19: Stromal-Epithelial Cytokine Crosstalk in Experimentally Induced Periodontal Disease

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Objectives: Lipopolysaccharide (LPS) is a bacterial virulence factor implicated in the conversion of junctional to pocket epithelium, an early marker of periodontal disease onset. During disease progression, the epithelial barrier is compromised, allowing virulence factors to insult underlying stroma. We sought to determine if LPS-induced changes in diffusible gene products could effect signalling crosstalk between stromal and epithelial tissues, contributing to disease.

Methods: Wistar strain rats (14 male) were divided between time 0 control and 8-week treatment groups. LPS was applied daily into the gingival sulcus and histological analysis confirmed the onset of disease. Junctional epithelium and underlying stromal tissues were separately collected from healthy and diseased animals by laser-capture microdissection and subject to gene expression microarray analysis. Genmapp bioinformatic analysis was performed to identify gene ontology function groups of high significance (z > 4) whose protein products could potentially interact. In vitro validation used a chronic wound cell culture model and protein analysis by flow cytometry.

Results: LPS-altered gene ontology function grouping top-ranked the molecular binding category in both epithelia and stromal tissues. However, for stroma, the cytokine subgroup ranked near the top (z=5.991). Its three top-ranked stromal genes (amphiregulin, interleukin 1-β, and Fas ligand) are known to be diffusible and capable of modulating the epithelial growth factor (EGF) pathway. For epithelia, several binding subgroups associated with the EGF receptor were highly ranked, including ErbB-2 class receptor binding (z=4.994). Its top three altered genes (Fos ligand, mucin 4, and somatostatin receptor) were downregulated. All are reported as playing a role in normally inhibiting EGF signalling. Upregulation of all 3 stromal and downregulation of all 3 epithelial gene products was confirmed in vitro for up to 3 weeks with LPS treatment.

Conclusions: LPS may contribute to the onset of periodontitis by upregulating EGF pathway activity via stromal-epithelial crosstalk.

Acknowledgements: Supported by Canadian Institutes of Health Research grant MOP-82830 to EEP.
Abstract #20: Role of WNT11 During Avian Facial Morphogenesis

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Objectives: Wnts are secreted glycoproteins and there are 19 in mammals. Little is known about the role of Wnts in facial morphogenesis. We recently found that WNT11 is expressed close to the lip fusing regions. Here, we investigated the hypothesis that WNT11 regulates the process of lip fusion.

Methods: The mechanism for the clefts is determined by examining the effects on target genes in WNT11 RCAS injected embryos. Other techniques include transfection, cell culture, chemicals injection, bead implantation, and double and single in situ hybridization.

Results: In a recent screen for the expression of many Wnt signalling pathway genes in the face, we found that WNT11 is first localized in the cranio-medial of the maxillary prominence. Later WNT11 expression is shifted out of the fusion zone and is restricted to the lateral mesenchyme under the eye. WNT11 is never expressed in the frontonasal mass and thus might act as a negative regulator of lip fusion. In the present study, we found that misexpression of WNT11 in the maxillary prominence/frontonasal mass using an avian retrovirus leads to large gaps in the soft tissues and skeleton. Recently WNT11 polymorphisms have been found in patients with clefts. We also found that WNT11 overexpression downregulates the expression of MSX1 and DLX5 whereas upregulates SOX9 and DKK1 (canonical Wnt antagonist). Further we also found that SHH, BMP4, and FGF8 negatively regulate WNT11 expression whereas RA induces WNT11. These results are the first to show the context-dependent regulation of WNT11 and its interaction with the other known signalling pathways involved in normal facial development.

Conclusions: Thus, we identified WNT11 as a new gene involved in facial clefting. Detailed study on this will reveal new aspects of facial development which could be used to treat craniofacial defects.

Acknowledgements: This study was supported by a post-doctoral fellowship from the Michael Smith Foundation for Health Research to PGL.
Abstract #21: Implant Surface Roughness Modulates Macrophage Morphology and FAK-MAPK Signalling

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Implant surface topographies are known to alter cell morphology and subsequent cellular behaviour in vitro and in vivo. Upon implantation, macrophages are among the first cell types to attach to the implant and their secretions are essential for wound healing. Previous studies have shown that rough surface topographies increase secretion of pro-inflammatory cytokines (IL-1, IL-6, and TNF-α) by LPS-stimulated macrophages. The hypothesis underlying this study was that culturing the topographies of different roughness would differentially activate signalling pathways associated with cytokine secretions.

Objectives: Our aim was to study the effects of implant surface topography on RAW 264.7 morphology and the FAK-Src and ERK1/2 signalling cascade involved in cytokine secretion.

Methods: Macrophages (RAW 264.7) were seeded on Ti-coated epoxy replicas of polished, blasted, etched, and blasted & etched (SLA) surfaces. SEM, immunocytochemistry, and Western blotting techniques were used to analyze the effects of surfaces. The Src-specific inhibitor PP1 was used to test the linkage between the above intracellular signalling molecules.

Results: Macrophage spreading on polished surfaces significantly increased with time but on rough surfaces was significantly decreased after one day (ANOVA with Bonferroni, p<0.05). Macrophages on SLA showed increased Src and ERK1/2 phosphorylation compared to polished surfaces at day 1 while at day 3, FAK, Src, and ERK phosphorylation was decreased two-fold by SLA compared to polished surfaces. The inhibition of Src activation using PP1 caused a downregulation of FAK, Src, and ERK1/2 phosphorylation on all surfaces (ANOVA with Bonferroni, p<0.05), indicating a linkage between the three molecules.

Conclusions: Implant surface topography affects signalling pathways known to control cytokine release and, as a result, may modulate wound healing around implants.
Abstract #22: Pinoy Smiles: A Dental Education Website Developed with Community Participation

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Previous research by the Healthy Teeth Healthy Families group has linked the increased susceptibility of Filipino-Canadian children to early childhood caries with limited caregiver knowledge regarding the care of young children’s teeth. The paucity of culturally appropriate materials was cited as one of the main barriers hindering access to dental information. The Internet was suggested as a viable tool to deliver ethno-specific dental information.

Objectives: To develop an interactive website for delivery of information regarding the dental care of young Filipino children, in consultation with the Filipino-Canadian immigrant community of Metro Vancouver.

Methods: Formative evaluation of content, design, and format was undertaken using qualitative methods involving interviews with a sample of Filipino immigrant parents and childcare providers. The website was designed to include a number of features that are known to increase the effectiveness of health information delivered via digital media and which have been cited by our past research participants as particularly desirable.

Results: Based on formative evaluation, the website’s final main content included short informative articles with colourful graphic images, a question & answer section, colouring pages for children, a smile gallery, and a video sing-a-long. To increase website and viewer interactivity, comment forms were enabled after posts and a “Smile of the Month” contest using on-line polls was created. Survey participants indicated that the website was culturally appropriate and that the information was presented in a desirable manner (including length, visual appeal, and language level).

Conclusions: Continual community involvement, feedback, and consultation during the design of a dental education website are recommended to produce culturally appropriate and appealing health educational materials for minority populations such as Filipino-Canadians.

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Abstract #23: Peripheral Calcifying Epithelial Odontogenic Tumour: Case Report and Literature Review

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Calcifying epithelial odontogenic tumour (CEOT or Pindborg tumour) is a rare odontogenic tumour with unique clinical, radiological, and histological features. It can have both central (intraosseous) and peripheral (extraosseous) types. The latter type is extremely rare and can be a challenge in histological diagnosis.

Objectives: The objectives of this study are (1) to report a rare peripheral type of CEOT with challenges in radiology and pathology and (2) to review the literature and update the current findings of this rare disease entity.

Methods: This is a case report of a painless, slow-growing gingival mass at the anterior labial gingiva with detailed documentation using clinical images, computed tomography, and microimages. An updated review of the literature has also been provided.

Results: A 52-year-old male patient presented with a 3-cm swelling mass at the anterior labial gingiva for unknown duration. The mass was considered to be a radiographic defect from tumour growth; the presence of cellular atypia with areas mimicking glandular differentiation was such that the mass was once diagnosed as squamous cell carcinoma with ‘rare’ adenoid differentiation. Before a hemimaxillectomy procedure, a consultation with the BC Oral Biopsy Service was arranged. Upon the confirmation of immunohistochemistry studies using S100, Vimentin, CK7, and CK20 and a special stain of Congo Red, a diagnosis of a rare peripheral CEOT was confirmed. A conservative surgical procedure was performed.

Conclusions: This is a report of a rare peripheral CEOT case with a literature review. A correct diagnosis can save oral tissue from unnecessary removal and, consequently, reduce morbidity and improve quality of life.
Abstract #24: G9a Positively Regulates Osteoblast Differentiation

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Objectives: Epigenetic controls by histone modification have a crucial role during development. One of the histone modification enzymes, G9a, methylates lysine 9 of Histone 3, which inhibits transcription. Since germline mutations of G9a cause embryo death by E10.5, the function of G9a during development has not been fully explored. To overcome this issue, we generated mice with a conditional inactivation of G9a only in neural crest-derived cells. The objective of this study was to characterize the function of G9a during osteoblast differentiation.

Methods: Wnt1-Cre deleter strains were crossed to the floxed allele of G9a. Correctly targeted embryos have deletion of G9a only in neural crest-derived tissues and this was confirmed with G9a monoclonal antibodies. All mice were injected with BrdU 2h before euthanasia for subsequent cell proliferation analyses. Mineralization of bone and cartilage was examined using microCT scan and wholemount Alcian Blue and Red staining. For histology, immunofluorescence, BrdU, and apoptosis, serially sectioned tissues were used. The extent of osteoblast differentiation was confirmed by alkaline phosphatase activity, Osteopontin, and Twist1 expression.

Results: The Wnt1-Cre/G9afl/fl mice were noticeably smaller than wild-type mice one week after birth. MicroCT and skeletal staining showed there were numerous holes in the neural crest-derived bones, however cartilage elements appeared normal. The bone loss was correlated with decreased proliferation of osteoblastic cells in Wnt1-Cre/G9afl/fl mice, however there was no increase in apoptosis. Furthermore, at E14.5, Wnt1-Cre/G9afl/fl mice showed a delay in expression of Osteopontin, which is already present in wild-type mice. An early marker of the osteogenic front, Twist1, was retained for a longer period of time in Wnt1-Cre/G9afl/fl mice compared to the wild-type embryos.

Conclusions: Repression of gene transcription by G9a promotes differentiation of osteoblasts. Wnt1-Cre/G9afl/fl mice have a delay in osteoblast commitment/differentiation and these defects are caused in part by reduced proliferation.

Acknowledgements: This study was supported by research grants from the Canadian Institutes of Health Research to FMR and JMR.
Abstract #25: Ongoing Investigation of DHDP Degree Completion Students and Graduates

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Objectives:

Methods:

Results:

Conclusions:

Acknowledgements: UBC Faculty of Dentistry Undergraduate Summer Research Student Award.
Abstract #26: Characterization of Progenitor Cells from the Submandibular Salivary Gland

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Objectives: Radiation therapy for cancer in the head and neck areas and the autoimmune disease Sjögren’s syndrome are the main causes for loss of salivary gland function, which leads to xerostomia. The symptoms of xerostomia include dry mouth, dysphagia, dental caries, and oropharyngeal infections. Currently, there is no effective therapy to restore the function of affected salivary glands. Stem or progenitor cells that are able to regenerate defective organs have been found in various tissues, providing a promising way to restore organ function. We hypothesized that the mouse submandibular gland contains progenitor cells that can be isolated, maintained, and propagated in culture while maintaining their differentiation potential for salivary gland epithelial cells.

Methods: The putative progenitor cell subpopulations were isolated from adult male mouse submandibular salivary glands and propagated in culture. Two clonogenic cell populations with different cell morphology were further characterized using monolayer and three-dimensional (3D) culture systems, phase-contrast and electron microscopy, immunostaining, and quantitative real-time PCR.

Results: We isolated, selected, and propagated two clonogenic, morphologically distinct subpopulations of salivary gland cells (spindle-like cells and epitheloid-like cells) and maintained them in culture for over 40 passages. When the epitheloid-like cells were placed in a 3D culture system, the cells differentiated to form acini-like spheroids that expressed the salivary gland acinar cell signature protein, amylase. In contrast, the spindle-like cells formed simple spheroid clusters in the 3D system and expressed signature proteins for myoepithelial cells of the salivary glands.

Conclusions: Mouse submandibular salivary glands contain progenitor cells that can be isolated, propagated, and selected based on clonogenic potential and cell morphology. The morphologically distinct cell subpopulations can differentiate in vitro to express proteins characteristic for acinar or myoepithelial cells. Further characterization of salivary gland progenitor cells will be helpful in tissue engineering aimed at regenerating salivary gland function.

Acknowledgements: Supported by the UBC Faculty of Dentistry.
Abstract #27: Securing a Dental Impression: To Lubricate or Not, That is the Question

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The clinical motivation was that Elastomeric Impression Materials (EIM) may apply adverse extrusion forces on periodontally involved teeth and implant-supported splints. A patient incurred major trauma to an existing implant supported by a full arch prosthesis during routine dental impression taking.

Objectives: Our objective was to test the hypothesis that lubrication decreases the force required to remove set elastomeric impression materials (EIM).

Methods: Five commercially available EIM were used: an inherently hydrophilic polyether (PE) [Impregum, 3M-ESPE]; three inherently hydrophobic poly-vinylsiloxanes (PVS) rendered hydrophilic by the addition of surfactants [Imprint, 3M ESPE; Take 1 Advanced, Kerr; VP Mix HP, Henry Schein Inc.]; and a new hydrophilic PE-PVS material [EXA’lence, GC]. Triad (Triad Disposables Inc.), a water-based oral lubricant, was used as well. A 10-mm diameter fibreglass rod, without and with lubrication, attached to the crosshead of an Instron 4301 universal testing machine, was inserted 25 mm into a 15 mm diameter test tube secured to the base of the machine and filled to a depth of 35 mm with freshly mixed EIM. After setting of the EIM, the rod was removed at a speed of 100 mm/min, simulating clinical conditions. Data acquisition software (System IX, Instron) was used to run the tests and collect force-displacement data. The maximum forces recorded during testing were analyzed using two- and one-way ANOVA, followed by the Bonferroni test for multiple means comparisons.

Results: The analysis revealed that both EIM and lubrication had a significant effect. Based upon the maximum force recorded during removal and irrespective of the absence or presence of lubrication, EIM were ranked as: Impregum > EXA’lence = Take 1 Advanced > VP Mix HP = Imprint. Our analysis also determined that lubrication significantly reduced (43 to 56%) the maximum force recorded for all EIM.

Conclusions: The null hypothesis was accepted. However, before recommending the use of lubrication in conjunction with taking dental impressions, its effect on impression accuracy and cast quality should be assessed.

Acknowledgements: UBC Faculty of Dentistry Undergraduate Summer Research Student Award.
Abstract #28: Composite Repair: Can Sodium Methoxide Help? A Pilot Study

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The clinical motivation of this study was to improve the repair of minor fractures that occur on resin composite (RC) restorations.

Objectives: Our objective was to test the hypothesis that pre-conditioning with sodium methoxide (SM) facilitates bonding of fresh RC to set and aged RC.

Methods: Parallel-epipedic blocks (6x6x15 mm) were made by incrementally packing a nano-hybrid RC (Grandio, Voco, Germany) into a mould and light curing each increment for 40 s. After de-moulding, the blocks were cured for 120 s and stored in 37°C water for seven days. A flat surface was exposed by wet grinding on 600 grit SiC. Three surface pre-conditioning procedures were used prior to bonding: rinse and dry (R&D) (control, Group C); treatment with a SM methanol solution for 60 s followed by R&D (Group M); and treatment with a SM acetone solution for 60 s followed by R&D (Group A). The treated blocks were placed in a mounting jig, covered with a lid carrying an elastomeric diaphragm delineating the bonding area, and ScotchBond Multi Purpose (3M ESPE), a dental bonding system, was applied following the manufacturer’s instructions. Fresh RC was packed into the diaphragm and light cured for 60 s. The lid and diaphragm were removed and the blocks with their bonded RC cylinders were stored in 37°C water for seven days. Each sample was then placed in the testing jig and tested for shear bond strength (SBS) using a computer-controlled (System IX) universal testing machine (Instron 4301). Results were analyzed using one-way ANOVA and Bonferroni t-tests for multiple means comparisons.

Results: Group A had significantly higher SBS than the other two groups, which were not significantly different from each other.

Conclusions: Under the conditions of this study, pre-conditioning with an acetone solution of SM was beneficial for RC repair. This procedure should be compared with those currently used.

Acknowledgements: UBC Faculty of Dentistry Undergraduate Summer Research Student Award.
Abstract #29: Dental Hygiene Baccalaureate Degree Education in Canada

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Objectives: This study aims to explore and analyze the experiences and outcomes of earning a dental hygiene baccalaureate degree from the perspective of diploma dental hygienists who have advanced their education to the baccalaureate degree level.

Methods: This study employs a qualitative phenomenological design using a maximum variation purposeful sampling strategy. After completion of a pilot study, several dental hygiene national and provincial associations helped recruit participants through an e-mail broadcast to their members. Hitherto, 13 semi-structured interviews have been conducted with dental hygienists who initially earned their dental hygiene diploma in Canada, subsequently practised dental hygiene for a minimum of two years, and then completed their dental hygiene baccalaureate degree from a Canadian university. Interviews were audio-recorded, transcribed verbatim, and coded for data analysis involving pattern recognition and thematic development.

Results: Preliminary findings indicate that the participants experience a broader education, being exposed to a wider scope of knowledge within and outside of dental hygiene theory, and a more independent learning environment with a heavier focus on literature review and critical thinking, compared to their experiences in their dental hygiene diploma education. Perceived outcomes associated with completing this degree include an increase in confidence, literature review and critical thinking ability, evidence-based decision making and integration into practice, comprehensiveness of clinical care based on an expanded knowledge base, credibility from professional colleagues and clients, and an appreciation for lifelong learning. Participants also commented on having more career opportunities available to them outside of the private clinical practice setting.

Conclusions: Exploring and analyzing dental hygienists’ experiences and outcomes of dental hygiene degree-completion education reveals important insights into the impact of earning a dental hygiene baccalaureate degree in Canada.

Acknowledgements: The researchers thank the Canadian Dental Hygienists Association, the British Columbia Dental Hygienists Association, the College of Registered Dental Hygienists of Alberta, and the Ontario Dental Hygienists Association for their assistance with participant recruitment.
Abstract #30: Clinical Reasoning in Dentistry: Across Levels of Expertise and Problems

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Objectives: To describe the process and strategies of clinical reasoning used by dental clinicians across different levels of expertise to develop a conceptual framework for curricular design and assessment of competency.

Methods: Using the “think-aloud” method, we interviewed 18 dental students about biopsychosocial problems identified in 6 vignettes; and 8 orthodontic residents plus 11 orthodontists about problems of craniofacial growth and malocclusion presented in 2 vignettes. The interview transcripts were analyzed to explore the process and strategies of clinical reasoning used by the participants.

Results: The reasoning process in both groups included: (1) a ritualistic approach to collect information for a treatment plan; (2) decision trees to evaluate treatment options and maximize the probability and utility of outcomes; (3) pattern recognition to recognize similar patterns in a situation; and (4) an integrated script of knowledge and experience triggered by related attributes of the script leading to a clinical diagnosis and plan. Six reasoning strategies (scientific, conditional, collaborative, narrative, ethical, and pragmatic) were used by both groups. However, experienced clinicians were more confident in their appraisal of uncertain situations and dilemmas as they integrated several reasoning strategies in the process, used refined scripts of knowledge and experience in familiar situations, and were able to reflect on the impact of their reasoning on the larger social, cultural, and political context.

Conclusions: Expertise in clinical reasoning develops through continuous framing and solving problems to refine networks of knowledge and experience.
Abstract #31: The Role of Periostin During Palatal Fusion

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Objectives: Epithelial mesenchymal transition (EMT) is considered to be involved in the disappearance of midline epithelial seam (MES) disintegration. Extracellular matrix components are important determinants in the cellular response to EMT inducers. TGF-β3 null mice that have cleft palates retain the basement membrane that is inversely correlated with the ability of MEE to transdifferentiate, suggesting that contact of MEE with mesenchymal matrix during palatal fusion may play an important role in promoting TGF-β3 mediated EMT. Periostin is a secreted extracellular mesenchymal matrix protein and exerts an influence on cellular reorganization. In this study, we investigated the role of periostin in EMT during palatal fusion.

Methods: The spatiotemporal expression pattern of periostin during palatal fusion was investigated by immunofluorescence and in situ hybridization. Confocal microscopic analysis was conducted to reveal the association between transdifferentiated MEE and periostin. The regulation of periostin was studied using real-time PCR.

Results: Periostin is expressed in the palatal mesenchyme with fine fibrillar networks and in the basement membrane, but not in the epithelium. However, during MES disintegration, periostin shows strong intensity in the basement membrane of MES and some MEE started to express periostin. Periostin in the basement membrane is mostly retained, even at stage E15.0, but barely seen at E16.0. Furthermore, a major epithelial matrix in basement membrane, laminin, is degraded earlier than periostin. Select transdifferentiated MEE are associated with periostin. Periostin mRNA in the palate is upregulated by treatment with TGF-β3, which mediates palatal shelf fusion. Periostin mRNA is decreased in palatal shelves treated with p38 inhibitor (SB20358) and PI3K inhibitor (LY294002), which represent signalling pathways linked to EMT.

Conclusions: Our data suggests that periostin may play an important role in palatal development as a potent inducible regulator of EMT in select cell populations undergoing cellular reorganization.
Abstract #32: Implant Treatment Outcomes at the UBC Graduate Periodontics Clinic: A Retrospective Analysis

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Objectives: Most implants have predictable outcomes and high survival rates. However, a small but significant subset of patients experience implant failure. A retrospective review of charts at UBC was conducted to determine how patient-, disease-, site-, surgeon-, and implant design-centered risk factors affect the survival of implants.

Methods: A review of implants placed between 1995 and 2006 was completed. Inclusion criteria required a one-year post-placement diagnostic radiograph. Bivariate analyses were used to identify variables associated with implant failure. Risk factors with p-values < 0.05 or that were deemed clinically relevant by previous studies were included in stepwise linear multiple regression and logistic regression analyses.

Results: Based on the inclusion criteria, 107 patients and 300 implants were included in the study. Follow-up ranged from 1.00 to 19.79 years (mean 4.08 ± 2.95 years). At the follow-up, 92.3% of implants survived and 84.1% of patients did not experience failure. In the failing implant group, 13.1% of patients had one failed implant and 2.8% of patients had two failed implants. The survival rate of replacement implants was 85.71%. Most factors studied had no statistically significant impact on survival. Only simultaneous sinus augmentation and removable prostheses were significantly associated with failure and guided bone regeneration was significantly associated with survival. In the regression analyses, the predictors showing the largest effect on thread exposure were model, jaw (in favour of mandibular implants), and surface (in favour of rough surfaces). The odds ratio for implant failure was 11.00 for sinus augmentation and 0.288 for decreasing implant width.

Conclusions: The survival rates for implants placed at UBC are similar to those reported in the literature. Most variables considered risk factors did not have a statistically significant effect on implant failure. Given the high survival rates of implants, the small sample size does not allow for trends in the data to reach statistical significance, even if a true association exists.
Abstract #33: How the Turtle Makes its Palate Without Palatal Shelves

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Objectives: Here, we studied for the first time the embryonic palate of the turtle Emydura subglobosa. We hypothesized that differences in expression profiles may explain the structural differences in the partly fused and partly unfused turtle palate. The objectives of our project were to first describe normal morphogenesis of the turtle secondary palate and then to examine expression of genes that are known to be important for maxillary morphogenesis in mammals.

Methods: Turtle embryos were obtained from the Toronto Zoo and incubated either at room temperature or at 30°C. Staging according to Yntema (1968) was carried out and embryos were collected between stages 11 and 19. Specimens were fixed in 4% paraformaldehyde, sectioned, and radioactive in situ hybridization was performed on serial sections. Neighbouring sections were used for cellular proliferation and cell apoptosis assays and routine histologic staining. Other embryos were stained for bone and cartilage to determine the ossification sequence of maxillary bones.

Results: Sections of stage 14 embryos showed that fusion of the upper lip had already taken place. The maxillary prominences were positioned at the lateral edges of the oral cavity, similar to other amniotes. By stage 16, maxillary bones had begun to differentiate but palatine bones had not started ossification. The most striking result was that no palatal shelves were observed at any of the stages studied. In snakes, birds, and alligators, palatal shelves bud off the maxillary prominences on the medial side, similar to mammals. Despite not forming palatal shelves, a palatine bone still differentiated beneath the nasal cavity at stage 18. Details of proliferation, apoptosis, and gene expression in the medial edge of the maxillary prominences will be presented.

Conclusions: The turtle secondary palate may not be evolutionarily homologous to the mammalian palate since it forms via different morphogenetic mechanisms and serves different functions.

Acknowledgements: This project was funded by an NSERC grant to JMR.
Abstract #34: The Roles of Wnt6 and Wnt4 in Intramembranous Bone Formation

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Objectives: Our aim was to use high density micromass cultures to recapitulate the process of intramembranous bone formation in vitro and then to study the effects of two Wingless-related proteins, Wnt4 and Wnt6, on skeletogenesis. We hypothesized that Wnt4 and Wnt6 would induce bone formation based on their expression in the facial epithelia and the recognized importance of epithelia in bone induction.

Methods: Micromass cultures containing mandibular mesenchymal tissue from stage 20-24 chicken embryos were incubated for 8 days in a mixture of conditioned media from cells expressing Wnt6 or Wnt4 and complete media (DMEM:F12, 10% FCS). Controls were treated with a mixture of conditioned media from the same cell line expressing Wnt6 and Wnt4, but not containing the expression constructs. Cultures were fixed in 10% formaldehyde and stained for bone and cartilage. Adobe Photoshop was used to quantify the area of bone, cartilage, and the culture. One-way ANOVA followed by Tukey’s post hoc testing (Statistica) was used to determine differences in matrix and culture size.

Results: Wnt6-treated cultures were significantly smaller and had significant reductions in bone and cartilage after the 8 day incubation period. In contrast, Wnt4 cultures were unchanged compared to controls. Wnt6 completely blocked differentiation of bone and cartilage at 1:1 to 1:5 dilutions. At 1:8, cartilage differentiation reappeared whereas bone was still significantly reduced. Normal bone and cartilage differentiated at 1:12 dilution.

Conclusions: Instead of promoting differentiation, during normal development, ectodermal Wnt6 in the face may act to inhibit bone and cartilage formation near the epithelium. Wnt4 had no effect on skeletal differentiation or the size of the cultures so this growth factor is less important for facial morphogenesis than Wnt6. In the future, antagonizing Wnt6 signalling could help to increase intramembranous bone during wound healing in the face.

Acknowledgements: SM was funded by a Health Professional Student Research Award from the Canadian Institutes of Health Research (CIHR); the project was supported by CIHR grants to JMR.
Abstract #35: Critical Role for αvβ6 Integrin in Enamel Biomineralization

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Objectives: Tooth enamel has the highest degree of biomineralization of all the hard tissues in the body. During the secretory stage of enamel formation, epithelium-derived ameloblasts deposit an extracellular matrix whose proteins are cleaved by enamelysin and kallikrein-4, resulting in almost total enamel matrix degradation. During this process, the ameloblast plasma membrane has direct contact with the matrix and its mineralizing crystallites. The receptors that mediate ameloblast-matrix adhesion and organization are not well characterized. Integrins mediate cell-matrix adhesion and signalling in most cell types. Therefore, we hypothesized that epithelia-restricted αvβ6 integrin plays a role in the organization of ameloblast matrix and biomineralization of enamel.

Methods: Sections of 12-day-old wild-type mouse mandibles were used to investigate the expression of αvβ6 integrin in ameloblasts of developing incisors using immunofluorescence and in situ hybridization techniques. Tooth histology from β6 integrin-deficient (β6/-) mice was analyzed and compared to the wild-type mice by light and electron microscopy. Enamel surface characteristics and mineralization were studied by scanning electron microscopy and microcomputed tomography. The ameloblast cell layer of incisors was extracted and analyzed by Western blotting for the expression of enamel proteins. Attrition of molars was investigated using a scale based on cusp heights.

Results: The maxillary incisors of the β6/- mice lacked yellow pigment and the mandibular incisors appeared chalky and rounded. Molars of β6/- mice showed severe attrition. Wild-type mouse ameloblasts expressed both β6 integrin mRNA and protein. Interestingly, ameloblasts of the β6/- mouse incisors abnormally accumulated extracellular matrix facing the forming enamel and within the ameloblast layer itself. Western blots confirmed that the accumulating proteins were mainly amelogenins as they are significantly overexpressed in the β6/- mice.

Conclusions: Integrin αvβ6 is expressed by ameloblasts where it plays a crucial role in regulating amelogenin deposition and subsequent enamel biomineralization.

Acknowledgements: Supported by grants from the Canadian Institutes of Health Research.
Abstract #36: Microarray and Proteomic Analysis of Breast Cancer and Osteoblast Co-Cultures

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Objectives: Proteases and in particular matrix metalloproteinases (MMPs) play a pivotal role in tumour metastasis through modulation of tumour growth, angiogenesis, and invasion. The cellular origin of these proteases is not always clear with both tumours and stroma contributing to the protease repertoire. Our goal is to characterize the interaction between metastatic breast cancer tumours and the bone microenvironment and the resulting changes in the protease repertoire.

Methods: We used an in vitro two-dimensional culture system in which the highly invasive human breast cancer cell line MDA MB 231 (MDA 231) and a sub-population 1833 (MDA 1833), derived by in vivo passaging with increased propensity for metastasis to bone, were overlaid onto a monolayer of differentiated osteoblast (MC3T3-E1) cells. The changes in the complete protease and inhibitor expression profile induced upon co-culturing of these cells were determined using the dedicated murine and human CLIP-CHIP™ microarrays.

Results: An increase in MMP-13 mRNA expression was consistently observed when osteoblast cells were co-cultured with either MDA MB 231 or 1833. The elevation in osteoblast-derived MMP-13 was observed when the co-cultured cells were in direct contact, separated by filters, or when conditioned medium derived from the MDA MB 231 or 1833 was added, indicating the involvement of soluble factors. Changes in mRNA and protein expression were confirmed by QRT-PCR and Western blot analysis, respectively. Proteomic analysis using differential iTRAQ labelling and multi-dimensional liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) revealed changes in the osteoblast secreteme upon elevation of MMP-13 levels and several novel potential MMP-13 substrates were identified.

Conclusions: Our findings demonstrate the influence that metastatic breast cancer cells can have upon the osteoblasts, potentially manipulating the microenvironment to enhance the growth of metastases. Elucidating the dynamic relationship between breast cancer tumours and the microenvironment is essential to understanding this metastatic process.
Abstract #37: PI15 (Sugarcrisp) Modulates Patterning of the Avian Face

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Objectives: Our laboratory has shown that applying Noggin and Retinoic Acid (RA) to the early avian embryo causes a homeotic transformation in the face where the side of the beak is replaced by a duplicated set of midline elements. PI15 (Peptidase Inhibitor 15/Sugarcrisp) was one of the most highly induced genes that came out of a microarray experiment performed on the facial mesenchyme after RA/Noggin treatment. In this study, we investigated the hypothesis that PI15 is involved in face patterning.

Methods: The embryo manipulations that were mainly used in this study are retroviral injection, bead implantation, or the combination of both. Other techniques included transfection, cell culture, micromass culture, and double and single in situ hybridization.

Results: We found that PI15 is expressed in all of the regions of the face, but at older stages it is ultimately completely restricted to the frontonasal mass. We found that RA induced PI15 to a much greater extent than Noggin, suggesting that PI15 mediates the effects of RA, which validates the microarray analysis. Targets of PI15 were also identified; PI15 downregulated DLX5, MSX1, and MSX2. We also found that BMP4 and SHH induce PI15 expression whereas FGF8 inhibits it. Functions of PI15 in face development were identified by using PI15 retroviruses and resulted in inhibition of maxillary bone formation, which is half of the transformation process. The other half is the gain of midline skeletal elements. To check if PI15 can replace RA and synergize with Noggin to induce transformation, we overexpressed PI15 and simultaneously implanted a bead soaked in a 6-fold lower concentration of Noggin. As hypothesized, this induced a full set of duplicated elements.

Conclusions: The focus of this study identified a new gene that mediates the effect of the RA signalling pathway in controlling the jaw identity in the face.

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Abstract #38: The Virtual Articulator: Digital Casts with Dynamic Tooth Contact

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Digital dental casts, derived from impressions, conventional casts, or lately, intra-oral digitation, create a virtual environment for metrical analysis and fabrication of prostheses. Functional movements are rarely induced in these digital models despite their frequent application in dental articulators.

Objectives: We described a virtual articulator, designed with a new modeling platform (Artisynth), which can simulate dynamic collisions between digitized casts. The specific aim of the study was to determine how closely virtual tooth contacts matched those in conventional casts mounted on a semi-adjustable articulator.

Methods: This study included four steps: (1) mounting the casts in an intercuspal position on a Whipmix articulator; (2) recording cast positions, dental landmarks, and articulator parameters with a 3D Digitizer (Microscribe) and associated software (Rhino 4.0); (3) digitizing vinyl polysiloxane impressions of the casts (Orthocad, Cadent, USA); and (4) transferring digitized models and articulator parameters to Artisynth’s virtual articulator. Both the virtual and physical articulators produced terminal hinge jaw movements, midline protrusion, and laterotorusive excursions guided bilaterally with condylar and medial wall angles of 45 and 5 degrees, respectively. Static contacts in the intercuspal position, as well as dynamic contacts associated with anterior guidance, group-function, and cross-arch balance were compared qualitatively in the homologous virtual (marked digitally) and physical environment (marked with ribbon).

Results: In all cases, there was close correspondence between the zones of tooth contact in the virtual and physical casts.

Conclusions: The study demonstrates that valid collision-detection is possible in our virtual articulator. Moreover, Artisynth can import high-resolution models from any source, create kinematic motion from jaw-tracking records, and add viscoelasticity to model collisions. We suggest that with current technology it should be possible to develop a virtual articulator which does not require dental impressions or bite-records, and which incorporates tissue elasticity on tooth contact.

Acknowledgements: UBC Faculty of Dentistry Undergraduate Summer Research Student Award.
Abstract #39: Dynamic Changes in Cranial and Facial Relations During Human Lip Development

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Introduction: Isolated cleft lip, a common human congenital craniofacial malformation, can be caused by deficient or delayed outgrowth of the facial prominences limiting their contact and fusion.

Objectives: The purpose of this study was to analyze spatial and temporal relations between key structures and potential signalling sites in serial sections and 3D models during development of the primary palate (PP; midface, nose, and lip).

Methods: Transverse and coronal sections of human embryonic heads were reconstructed with the program “WinSURF” to illustrate relations between cranial and facial structures such as the brain, eyes and optic stalks, nasal pits and olfactory epithelium, the medial nasal prominence (MNP), developing adenohypophysis (AH), diencephalon floor (DF), trigeminal ganglia (TG), and the facial prominences (FP).

Results: During PP formation, lack of definitive boundaries between the face and brain permits close association between facial, cranial base, and cranial tissues. In stage 16 and early 17 embryos, the DF lies within the MNP and extends to the roof of the oronasal cavity. In the midline, a large ectoderm thickening (developing AH) is present between the DF and the midbrain. Nasal pits and eyes are laterally positioned and large TG lie immediately behind the eyes. In advanced stage 17 and 18 embryos, the DF is elevated, the MNP narrows and increases in height, and maxillary prominences grow forward to contact the MNP. Closure of the PP must occur while epithelium is capable of fusion and before these growth patterns displace components. Analysis of an embryo with bilateral cleft lip revealed asymmetric and reduced growth of the DF and AH due to the presence of a blood clot in the area.

Conclusions: Components of the developing face and developing brain are first closely associated and then separated. During lip formation, abnormal function of signalling sites could delay outgrowth of FP and delay fusion.

Acknowledgements: Collection of the material for this study was supported by Grant MT 4543 from the Medical Research Council of Canada. NP was funded by a CIHR Health Professional Student Research Award.
Abstract #40: Role of Proteolysis in Platelet Storage Lesion: Connecting Proteases to their Substrates

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**Objectives:** Platelets are a critical component of blood which aid in blood clotting. Donour-derived platelet concentrates are most commonly used to support burn victims and patients with severe bleeding conditions. During storage, platelets undergo poorly characterized changes defined as the platelet storage lesion (PSL). PSL is detrimental to their post-transfusion clotting function and limits their storage time to 5-7 days. The literature suggests that proteases are involved in PSL initiation and progression, however their precise role remains unknown. Our study investigated the molecular mechanisms of protease involvement in PSL by monitoring functional (using *in vitro* biochemical assays) and molecular (using proteomics) changes in platelets stored with or without protease inhibitors.

**Methods:** We employed a novel proteomic screen where the samples were isotopically labelled at the protein level (enabling quantification) and enriched for N-terminal parts of each protein (allowing characterization of proteolysis-derived neo-N-termini and protein original N-termini). This screen, termed TAILS (Terminal Amine Isotopic Labelling of Substrates), was employed to characterize platelets stored for 9 days with or without a protease inhibitor cocktail.

**Results:** We have identified 759 peptides corresponding to 466 proteins (compared to 1,160 proteins without N-terminal enrichment). These peptides include 401 original N-termini and 358 proteolysis-derived peptides. Protease inhibitor incubation caused a >20% decrease in the amounts of 121 peptides. Further, specific protease inhibitors were used to connect each protease class with its own subset of substrates.

**Conclusions:** Our preliminary studies identified matrix metalloproteinases as detrimental and serine proteases as supportive proteases of platelet function, thus suggesting new avenues for maintenance of platelet quality at longer storage periods.
Abstract #41: Extracellular Matrix Proteoglycan Degradation by Fibroblast and Macrophage Metalloproteinases

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Proteoglycans of the extracellular matrix are important regulators of cell migration, growth factor/chemokine localization, and matrix assembly in development, homeostasis, and disease processes. The proteoglycan versican is synthesized by proliferating myofibroblasts in wound healing and in the stromal reaction to cancer. Associations between versican content and cancer severity have been shown for a number of cancers, including oral cancer. Versican is anti-adhesive and is part of an encapsulation reaction to malignant cells. Thus versican degradation may facilitate cancer progression.

Objectives: To determine whether fibroblast and macrophage metalloproteinases are responsible for physiological versican turnover.

Methods: We have developed cell culture and biochemical models to determine how versican is degraded by fibroblasts and by wound healing/tumour stroma macrophages. Versican was purified from human fetal lung fibroblasts. Engineered versican domains and metalloproteinases were cloned and expressed in E. coli for degradation experiments. A novel mass spectrometry technique was used to determine cleavage sites in versican.

Results: The plant lectin concanavalin-A (ConA) induced a degradative phenotype when added to fibroblasts in vitro; this increased expression of MMP2 and MT1MMP (by protein, zymogram, and mRNA analysis) and a concomitant loss of versican from the pericellular matrix. Recombinant MMP2 and MT1MMP degraded purified versican in vitro. MT1MMP had the most restricted activity of any MMP tested, but cleaved in the G3 domain of versican, which we have predicted to be important in versican polymerization, and that others have suggested may be involved in cell-matrix interaction. The macrophage metalloproteinases MMP7 and MMP12 had the greatest activity against versican, cleaving at multiple sites.

Conclusions: Degradation of versican by macrophage and fibroblast enzymes is part of a normal “wound healing” progression. MMP7 and MMP12 released by macrophages are powerful versican-degrading enzymes. Inhibition of these activities could enhance the stromal encapsulation reaction and modulate the process of cancer invasion.
Abstract #42: Integrated Clinical Care and its Impact on Undergraduate Dental Radiology Teaching

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Objectives: The purpose of this study was to evaluate the number of intraoral radiographs that were taken per student and per month throughout the course of an academic year. Statistical analysis was done to better understand the standard number of radiograph interpretations required for student competency and to predict instructor demands in the integrated clinical care (ICC) environment.

Methods: All patient intraoral radiographs taken during the 2008/09 academic year were attained without data loss using the Planmeca Romexis report program. Microsoft Excel was used to mine data. Array formula and frequency functions were used to determine the number of intraoral radiographs taken by each student and the number of radiographs each patient received. Clinical procedure value data was used to understand student productivity during ICC. Statistical analysis was performed using SPSS 16.2 software. Descriptive statistics showing mean, median, minimum, maximum, and standard deviation was accomplished. Spearman correlations with a p value of < 0.05 were considered statistically significant.

Results: During the academic year, 8,611 intraoral radiographs were taken. The average number of intraoral radiographs per month was 956. The minimum occurred in December with 443 and the maximum occurred during February with 1,495 intraoral radiographs taken. The average number of radiographs taken per student in 3rd and 4th year was 47 and 143, respectively. 6.8% of patients received full mouth surveys. The number of radiographs taken per student did not correlate with the student’s number of procedures based upon statistical analysis.

Conclusions: We understand that this number may vary, but it is probably very important that students take and interpret at least 18 and 96 intraoral radiographs in 3rd and 4th years, respectively. The demand for ICC instructors was highest in February, followed by April, September, November, October, and March. The lowest demand for radiograph interpretation occurred in January and December.

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Abstract #43: General Dentists in British Columbia and the Child Patient

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Objectives: In British Columbia, general dentists in private practice provide the majority of dental care to children. The goal of this research was to explore the complex issues related to the provision of dental treatment to children by BC’s general dentists.

Methods: A web-based survey of the 343 general dentists who identified themselves in 2009 on the BC Dental Association’s referral database as willing to treat children was used. Prior to developing the survey instrument, 6 pediatric dentists were interviewed using a semi-structured interview guide about the challenges of treating children in today’s society. The final survey instrument, developed from information gathered at these interviews, was then pre-tested with a group of general dentists and submitted for ethics approval.

Results: The pediatric dentists mentioned several issues of concern when general dentists provide pediatric dental care. They discussed the potentially harmful behaviour patterns that may occur after a failed treatment appointment at a general dentist’s office and the difficulty to recondition a child after this previous negative experience. Failed restorations done by general dentists result in problems with reimbursement by insurers when these children are referred onto specialists for continuing care. Furthermore, parents are often misinformed by general dentists about various aspects of the treatment that will eventually be done at a specialist’s office. As a result of the information from these interviews, a multi-item questionnaire was developed that included the challenges of treating children, behaviour management concerns, treatment with sedation/GA, and communication barriers between general dentists and specialists.

Conclusions: Seeking input from pediatric dentists about the challenging issues facing today’s practitioners who treat children resulted in a robust and relevant survey instrument. Results of the survey will be reported in early 2010.

Acknowledgements: This study was supported by a Dentistry Canada Fund grant.
Abstract #44: Specially-Fed ApoE-Deficient Mice Reveal a Pathology Similar to Lung Sarcoidosis

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Objectives: Sarcoidosis is a chronic disease of unknown etiology characterized by the formation of non-necrotizing epithelioid granulomas in various organs, especially in the lungs. The lack of an adequate animal model reflecting the pathogenesis of the human disease is one of the major impediments in studying sarcoidosis. In this report, we describe Apoe-/- mice on a cholate-containing, high fat diet which exhibit granulomatous lung inflammation similar to human sarcoidosis.

Methods: Apoe-/- mice were fed for 19 weeks with a cholate-containing, high fat diet and their lungs were analyzed by immuno- and histochemistry after 8, 16, and 19 weeks.

Results: Histological analysis revealed well-defined and non-necrotizing granulomas in about 40% of mice, with the highest number of granulomas after 16 weeks on a cholate-containing, high fat diet. Granulomas contained CD4⁺ and CD8⁺ T cells and the majority of the cells in granulomas showed immunoreactivity for the macrophage marker Mac-3. Cells with morphological features of epithelioid cells expressed angiotensin-converting enzyme, osteopontin, and cathepsin K, all characteristics of epithelioid and giant cells in granulomas of human sarcoidosis. Giant cells and non-specific inclusions such as Schaumann's bodies and crystalline deposits were also detected in some lungs. Granulomatous inflammation resulted in progressive pulmonary fibrosis.

Conclusions: The observed similarities between the analyzed mouse lung granulomas and granulomas of human sarcoidosis, as well as the chronic disease character leading to fibrosis, suggest that this mouse model might be useful in the study of sarcoidosis.

Acknowledgements: This study was supported by a research grant from the National Institutes of Health.
Abstract #45: Does Oral Cancer Remain a Deadly Disease in British Columbia?

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Objectives: Oral cancer is a deadly disease with high morbidity and mortality globally. In BC, there is no reported outcome data regarding staging and treatment. A recent publication describes a novel approach using direct fluorescence visualization (FV) technology in the operating room to significantly reduce the local recurrence rate from 25% to 0%. The objectives of this study were to (1) gather historical data prior to the implementation of FV into treatment and (2) compare the time to local recurrence prior to and after FV usage.

Methods: Oral cancer cases were identified from the population-based BC Cancer Registry between January 2000 and December 2003. The selection criteria included those with a diagnosis of squamous cell carcinoma and carcinoma in situ, located at the anterior two-thirds of the tongue, floor of the mouth, palate/soft palate complex, gingiva, and the buccal mucosa where the FV can be applied.

Results: Over a 4-year period, 340 cases were selected from 686 head and neck cancers. The average age was 65.4 ± 13.2 years and 58% were male. The majority were from high-risk sites, including the tongue (N=129) and the floor of the mouth (N=76). Surprisingly, 41% were late-staged diseases and the cumulative 5-year disease-free survival rate was 40%. For 324 cases with follow-up information, Stage III/IV diseases had significantly poor prognoses in local control or disease-related deaths compared to those at early stages (66% vs. 47%, \(P=0.003\)). Surgery remains the treatment choice, especially for early-staged diseases. Among those T1 and T2 diseases (N=108) treated with intent-to-cure surgery only, 40% recurred. This was much higher compared to those after the implementation of FV in BC (12%; \(P<0.0001\)).

Conclusions: In BC, oral cancer is often diagnosed at a late stage. The introduction of novel FV technology has provided a more effective modality to improve local control of this disease.
Abstract #46: Three-Dimensional Numerical Simulation of Root Canal Irrigant Flow with Different Irrigation Needles

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Objectives: Irrigation is an important phase of endodontic treatment. The exact parameters that contribute to the effectiveness of various irrigation needle tip designs remain uncertain. The purpose of this study was to investigate, using computational fluid dynamics (CFD), the effect of needle tip design on irrigant flow pattern.

Methods: Parameters of an in vitro irrigation model were used to create CFD models. Experimental data obtained by recording the dynamic fluid distribution during irrigation with gauge 27 notched (Appli-Vac) and side-vented open-ended (Vista-Probe) needles, placed at 3 mm and 5 mm from the apex of a simulated straight root canal prepared in a plastic block, were used to validate the results of CFD analysis. Two “virtual” needle tip designs were also included in CFD analysis: one with a bevelled tip (based on Appli-Vac) and one with a side-vented closed-end tip (based on Vista-Probe). Apical pressure, flow velocity at wall, and flow velocity distribution within the root canal were determined by CFD.

Results: Flow patterns generated by CFD were in close agreement with the in vitro model. When placed 3 mm from the apex, the irrigant reached, or almost reached, the apex with all four needle designs. When placed 5 mm from the apex, the irrigant did not reach the apex with the side-vented needles. Irrigant velocities on canal walls were very low (0-0.7 m/s) compared to that within the needle lumen (~7 m/s) and varied as a function of needle tip design. Apical pressure was highest with the bevelled needle and lowest with the side-vented closed-end needle.

Conclusions: Irrigation needle tip design influences flow pattern, flow velocity, and apical wall pressure, all important parameters for the effectiveness and safety of irrigation. CFD can be a valuable tool in assessing the implications of needle tip design on these parameters.

Acknowledgements: This study was supported in part by a Pilot Project Award from the UBC Faculty of Dentistry.
Abstract #47: MMP Processing of Monocyte Chemoattractants CCL15/CCL23 Results in Increased Agonist Activity

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Objectives: Chemokines are chemoattractant cytokines that mediate directional leukocyte migration in innate and acquired immunity. Hence, the regulation and termination of chemokine activity is critical to control of the inflammatory response. The Overall laboratory established chemokines to be substrates of matrix metalloproteinases (MMPs), thereby modifying receptor binding, activation, and inflammation in vivo. To further understand the control of monocyte recruitment, we used a family-wide approach to evaluate MMP processing, and thereby function modification, of all CC chemokines involved in monocyte attraction.

Methods: In vitro cleavage of 14 chemokines by 9 MMPs that we expressed and purified was detected and confirmed by matrix-assisted laser desorption–time-of-flight mass spectrometry and Tris-tricine polyacrylamide gel electrophoresis. All 14 chemokines were susceptible to cleavage by one or more MMPs. A monocytic and a chemokine receptor-transfected cell line were then used to evaluate the functional effects of MMP-processing of CCL15 and CCL23. Receptor activation and the chemotactic capacity of the truncated chemokines were assayed by calcium flux and Transwell migration, respectively.

Results: These in vitro assays indicated that amino-terminal truncation of CCL15 and CCL23 by MMPs results in stronger receptor agonists, and up to one order of magnitude more than for the CCL7. To determine the potential role of these chemokines in human arthritis, we found by using protease inhibitors that both MMPs and a serine protease in arthritic synovial fluid process CCL15 at two separate sites. A proteomics approach has also been employed for the identification and quantification of full-length and truncated chemokine to confirm the in vitro findings.

Conclusions: These results further implicate MMP processing as a means of regulating chemokine function and innate immunity. Moreover, the increased agonistic activity observed by CCL15 and CCL23 processing suggests a role for proteolysis of these chemokines in monocyte recruitment, macrophage activation, and propagation of inflammatory responses.
Abstract #48: Improved Killing of Mixed Plaque Bacteria by Modified Photoactivated Disinfection

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Objectives: Photoactivated disinfection (PAD) by the combined use of light and photosensitizer has been proposed as an effective treatment of local bacterial infections. This study compared the effectiveness of conventional and modified PAD in killing mixed plaque bacteria.

Methods: A bacteria suspension from mixed plaque (cfu 1x10^8/ml) was incubated with six different solutions as follows: Methylene Blue (MB, 30 µM); MB mixed with 0.1% Chlorhexidine (CHX) and hydrogen peroxide in concentrations of 1%, 0.1%, or 0.01%; MB mixed with 0.1% CHX and 0.1% or 0.01% EDTA. After five minutes, the bacteria in the solutions were exposed to Laser Irradiation (Diode Laser, MM Optics, 40 mW, 660 nm). Samples were taken after 0.5, 1, and 3 minutes of exposure and serially diluted and cultured anaerobically on rich blood agar plates for 72 hours. Colony forming units were calculated from three parallel experiments. Bacterial survival was evaluated by comparing to negative controls.

Results: Conventional PAD with MB showed the poorest efficacy against mixed material suspension; after 3 minutes of radiation, 1.19% bacteria were still alive. Addition of low concentrations of CHX and EDTA to MB greatly enhanced the PAD effect, resulting in complete eradication of the bacteria in 30 seconds. Use of hydrogen peroxide in the mixture instead of EDTA also enhanced the killing considerably; however, ca. 0.02% of the bacteria were still alive after 30 seconds of laser irradiation.

Conclusions: The modified PAD was superior to conventional PAD at each experimental time used in the study. Addition of 0.1% CHX with either EDTA or hydrogen peroxide to Methylene Blue followed by laser irradiation resulted in complete or nearly complete eradication of all bacteria in just 30 seconds. The enhanced killing may be partially caused by improved penetration of the substances into the bacterial cells due to synergistic action on the cell wall permeability.
Abstract #49: P16INK4A Immunoexpression: A Negative Predictive Marker for High-Risk HPV in Oral Precancers

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Studies have shown a strong correlation between high-risk HPVs (HPV-HR) and a subset of head and neck cancer with a better survival outcome. Currently there is no gold standard or “lab friendly” markers for HPV detection in head and neck cancers. Due to the availability and readiness, immunohistochemical detection of p16 has been used widely in pathology laboratories as a surrogate marker for the presence of HPV-HR in cervical cancer and precancers, but not for those at oral and oropharyngeal sites.

Objectives: (1) To explore the presence of HPV-HR in precancers of oral and oropharyngeal sites using chromogenic in situ hybridization (CISH) and (2) to correlate the presence of p16 and HPV-HR.

Methods: A total of 205 oral mucosal lesions diagnosed as cancer, precancer, and normal (N=24) were analyzed for HPV-HR (cocktail for types 16/18/31/33/35/39/45/51/52/56/58/59/68 and HPV16/18) using CISH and for p16 using immunohistochemistry.

Results: Fourteen percent of cases showed the presence of HPV-HR with site predilection (28% soft palate complex vs. 13% anterior mouth, P<0.0001). Half of the HPV-HR cases were positive for HPV16/18. Severe dysplasia/carcinoma in situ (HGD) samples showed the highest rate (37%) of detection of HPV-HR, compared to 5% of low-grade dysplasia and 10% of invasive cancer. Of these HGD, 57% were located at the soft palate complex. Forty-five (25%) cases showed large patchy or diffuse cytoplasmic overexpression of p16. The sensitivity, specificity, positive predictive value, and negative predictive value of using p16 as a surrogate marker for HPV-HR were 0.92, 0.92, 0.66, and 0.99, respectively.

Conclusions: This is the first and largest series of oral tissue microarrays to detect the presence of HPV in oral dysplasia. The presence of HPV16/18 does not explain those without obvious risk factors in this study set. Patchy or diffuse patterns of p16 immunostaining can provide an easy, negative marker for the presence of HPV-HR.

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Abstract #50: Evaluation of Canal Instrumentation Using GT Series X™ versus Prosystem GT™

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Objectives: The ability of two nickel–titanium rotary file systems, GT series X™ and Prosystem GT™, to maintain the original path of the canal were compared to original canal location using a split-mould design (the endodontic cube).

Methods: The mesial roots of 32 mandibular first and second molar teeth were selected with separate canals from orifice to foramen and curvature ranging from 15 to 40 degrees (total of 64 canals). The canals were divided randomly into two groups of 32 canals each. Working length for each canal was determined 1.0 mm short of where the file tip was visible on the external surface of the root under high magnification. Following access cavity preparation, each tooth was embedded in composite resin using the Endodontic Cube as a mould, before sectioning into 5-8 pieces based on the tooth length. After reassembly of the sections, canals were randomly assigned to receive either GT series X™ or Prosystem GT™ rotary instrumentation. Rotary instrumentation to size 30/06 using GT series X™ and Prosystem GT™ was carried out according to the manufacturers’ instructions. Digital photographs were taken of all of the sections before and after instrumentation, and Auto Cade software was used to measure canal enlargement and movement of canal centers by superimposing the images of the instrumented and non-instrumented canals.

Results: Preliminary results indicate that normalized canal widening using GT series X™ files is 23% compared to 42% using Prosystem GT™ files. Normalized center displacement using GT series X™ files is 16% while in the Prosystem GT™ group it is 26%.

Conclusions: Both systems tended to move the original canal toward the furcation, but the Prosystem GT™ caused greater canal transportation.

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Abstract #51: Investigation of Tobacco-Cessation Barriers in Patients with High-Risk Lesions

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With tobacco and alcohol risk behaviours contributing to three-quarters of cases, oral cancer is recognized as a preventable disease. Evidence shows that continuous smoking after treatment of high-risk oral lesions (HRLs) is associated with recurrence risk. However, little is known about the impact of smoking behavioural changes in patients diagnosed with HRLs or their barriers for tobacco cessation.

Objectives: (1) To collect pilot data regarding tobacco-related behavioural changes prior to and after the diagnosis of an HRL and (2) to identify possible facilitators and barriers in tobacco cessation.

Methods: A survey-type questionnaire is being developed for collecting data on smoking behaviours and cessation barriers. Patients with ever-smoking histories within 5 years of an HRL diagnosis are invited to participate in the study. A pilot cohort (N=25) has been interviewed to develop a refined questionnaire for a larger scale study.

Results: Initial results show that all patients interviewed have a general understanding of the associations between tobacco consumption and its consequences on health. While 64% of the patients intend to or have successfully quit, 36% of patients are either undecided about quitting or do not intend to quit despite having an HRL diagnosis. Surprisingly, all but 2 patients have experienced difficulty in tobacco cessation and 16 (64%) of them experienced smoking recurrence after their attempts to quit. The HRL diagnosis was the strongest message for the patients who quit successfully. The identified cessation barriers were mainly stress and smoking enjoyment.

Conclusions: Incorporation of an open-ended interview questionnaire allows for better understanding of smoking behaviours and complex cessation barriers in patients with HRLs. The barriers to tobacco cessation appear to be more psychosocial in nature, i.e., stress and smoking enjoyment. Development of effective intervention strategies targeting these individuals is needed to improve tobacco cessation success, and consequently reduce the risk of HRL recurrence.

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Abstract #52: Opportunities for Community-Based Dental Clinics to Address Oral Health Inequalities

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Objectives: In Canada, dentistry is generally funded as an out-of-pocket expense rather than from the public healthcare system. Consequently, socioeconomic status influences access to dental treatment. A principal mandate of community dental clinics is to ensure access for vulnerable populations. The clinics have difficulty providing care at the reduced fees afforded by patients. The objective of our study was to collect information on community dental clinics in BC that provide dental services to economically disadvantaged communities to determine how their operations might be sustained and to inform business plans for organizations considering community-based dental care services.

Methods: This presentation provides evidence from a case study of five community dental clinics in British Columbia. Inclusion criteria were used to focus on one specific model of community clinic. These criteria were: operates as a non-profit organization; provides basic dental care services including preventive and restorative services; employs paid dental staff; operates full-time hours; and located in BC. Data collection included: (a) review of electronic files to collect aggregate patient and procedural data; (b) review of each clinic’s finances; and (c) open-ended interviews with key staff.

Results: The study demonstrated that, with modest financial support, it is feasible for community clinics to provide quality dental care services to vulnerable populations by reducing the financial barriers to accessing treatment while also providing care in integrated settings that are accessible and respectful. The standard of care accessible to the overall population can be provided efficiently within a community-based clinic. Specifically, for community responses to go beyond the limited mandate of relieving pain to include more comprehensive preventive, diagnostic, and restorative treatments.

Conclusions: There is significant potential to support the existing community dental clinics in BC and make evidence-based planning towards expanding these responses to improve access to dental care in other areas of the province.

Acknowledgements: This study was supported by a research grant from the British Columbia Medical Services Foundation (administered by the Vancouver Foundation).
Abstract #53: Unmasking the True Nature of Oral Lesions in a High-Risk Community

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Previous studies have shown a high prevalence of tobacco and alcohol consumption and high incidence of oral cancer (one cancer identified in 150 screened) in Vancouver’s Downtown Eastside (DTES). Thus, development of an effective oral cancer screening strategy in this high-risk community is urgently needed. We are currently evaluating several technologies for screening and early detection. Direct fluorescence visualization (FV) has shown high sensitivity for oral cancer or precancer detection; however, it also highlights other oral conditions commonly seen within the community setting such as oral infection and inflammation. We examined the potential use of imaging cytometry (IC) as an adjunct tool to FV for oral screening.

Objectives: (1) To measure altered cytological nuclear phenotype score (cNPS) using IC and (2) to assess whether IC can increase the accuracy in FV screening.

Methods: We collected 355 exfoliative cell samples using a small curved interdental brush from DTES residents between 11/2004 and 02/2009. Cytology slides were prepared, stained, and automatically scanned using the cyto-savant®. For each object (nucleus/debris) imaged, ~110 features were evaluated using cell recognition software to differentiate cells from debris.

Results: Of 355 brushings, 4 were from cancerous sites, 100 from non-cancerous common lesions (60, trauma; 28, inflammation; 12, infection), and 251 from normal mucosa. Using a previously trained algorithm with 84% sensitivity and 97% specificity to examine these samples, no difference between gender, age groups, smoking habits, and immune status (HIV infection) was observed. This algorithm correctly identified 3 of 4 high-grade lesions and 89% normal cases. For non-cancerous lesions at the FV loss area, cNPS assessment drastically increased accuracy from 10% to 83%, specifically, trauma, 13% to 90%; inflammation, 6% to 78%; and infection, 0% to 75%.

Conclusions: Pilot results support the use of combination direct FV and IC in oral cancer screening in a high-risk community.

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Abstract #54: Alterations in Tissue Autofluorescence Using Spectroscopy in High-Risk Oral Lesions

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Oral cancer can be difficult to identify early, even for trained specialists. New tools need to be developed to aid in screening patients within the community. Alteration in autofluorescence is one promising approach. Using spectrosopes to collect fluorescence spectra of alterations in tissue, autofluorescence has been used in cancer studies and has shown promising results in distinguishing cancer and normal tissue. There is a lack of information on reactive lesions, which are common in community settings.

Objectives: The objective of this study was to collect fluorescence spectra from normal mucosa, mucosa with high-risk histological change, and mucosa with chronic inflammation under different excitation wavelengths of light, and to compare the change in tissue autofluorescence of oral mucosal lesions between those with high-risk changes or inflammation.

Methods: Patients were recruited at the Oral Oncology Clinic of the BC Cancer Agency. Spectroscopic measurements were taken of lesional and normal sites using a fibre optic probe. Three excitation wavelengths were used: 436 nm, 405 nm, and UV. Differences in fluorescence loss of the emission peak between groups were compared using unpaired t-tests.

Results: Fluorescence spectra from precancerous/cancerous (N=16) and inflammatory lesions (N=8) were selected from 49 measured patients. Shorter excitation wavelengths elicit shorter emission wavelengths. A narrow emission range at 436 nm excitation suggests that it targets fewer fluorophores; in contrast, a broader range of peak emission under 405 nm excitation suggests a wider range of fluorophores targeted. When comparing the percentage loss of peak emission intensity (PEI), cancer cases showed more loss than dysplasias. Interestingly, there is a significant difference in percentage loss of PEI observed between dysplasia and chronic inflammation under 436 nm and UV excitation.

Conclusions: This is the first study to use 3 different excitation wavelengths to examine oral mucosa. This device has the potential to provide an objective, sensitive approach to be used within the community setting.

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Abstract #55: Oral Health Assessments for Elderly Residents of Long-Term Care Facilities

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Oral health is assessed in Canadian long-term care (LTC) facilities by nurses as part of the Resident Assessment Instrument – Minimal Data Set (RAI-MDS) Version 2.0, a computerized care-management tool. Although an exam by a dentist is the ideal method of assessing oral health, oral health assessments are important tools because non-dental professionals can perform them more frequently. Oral health assessments can identify dental problems, result in timely referral to dental professionals, and be used to develop and evaluate daily mouth-care plans.

Objectives: The aim of this research project was to perform a literature review to investigate how oral health is being assessed for elderly residents of LTC facilities in Vancouver, British Columbia.

Methods: A literature review from the dental and nursing literature was performed to identify articles related to oral health assessments for the geriatric population. Electronic searches using the PubMed and Google Scholar databases were conducted using combinations of the following terms: “geriatric,” “oral health,” “oral health assessment,” “assessment tool,” “nursing assessment,” “nursing oral care,” “Minimum Data Set,” “MDS,” “Resident Assessment Instrument,” “RAI,” “Inter-RAI,” “dental exam,” “long-term care,” “LTC,” “oral health exam,” “geriatric dentistry,” and “quality of life.” This search yielded 42 papers.

Results: From the literature review, several tested and reliable dental indices and oral health assessment tools were found. Dentists examine residents using indices that include the index of Clinical Oral Disorders in Elders (CODE), Oral Health Status Index (OHSI), and Oral Health Score (OHS). Nurses and care aides can be trained to use oral health assessment tools that include the Kayser-Jones Brief Oral Health Status Exam (BOHSE), Oral Health Assessment Tool (OHAT), and The Holistic and Reliable Oral Assessment Tool (THROAT).

Conclusions: The oral and dental sections of the MDS 2.0 do not assess oral health adequately and cannot generate effective care plans for daily mouth care. The MDS should be supplemented with evidence-based oral health assessment tools such as the BOHSE, OHAT, or THROAT.
Abstract #56: Integrin αvβ6 Loss Causes Enhanced Keratinocyte Proliferation and Retarded Hair Follicle Regression

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Objectives: Integrin αvβ6 is an epithelial-specific receptor that binds and activates latent transforming growth factor-β1 (TGF-β1). TGF-β1 has been implicated as an endogenous inducer of hair follicle regression during hair cycling. We found that β6 integrin knockout (β6−/−) mice exhibited an accelerated wound repair and increased the number of proliferating hair follicle keratinocytes compared to wild-type (WT) controls in a compromised wound healing model. This was associated with a reduced level of TGF-β1 activation. Therefore, we hypothesized that αvβ6 integrin-mediated TGF-β1 signalling regulates hair regeneration and the hair follicle involution process.

Methods: A standardized mouse model of depilation-induced hair cycling was established to trigger hair regeneration in WT and β6−/− mice. The hair cycle stages were assessed at different time points. The expression of αvβ6 integrin was studied in regenerating hair follicles. Catagen development was compared in the WT and β6−/− follicles. Keratinocyte proliferation as well as the expression, distribution, and activation of TGF-β1 were assessed.

Results: αvβ6 integrin was strongly upregulated during hair regeneration in WT follicles and its expression was hair cycle stage-dependent. β6−/− mice presented an accelerated hair regeneration compared to WT controls. The proliferating cell marker Ki67 immunostaining indicated that β6−/− follicles contained a significantly higher number of proliferating keratinocytes than WT follicles at the same stage. In addition, deletion of αvβ6 integrin caused retardation of hair follicle regression. β6−/− follicles displayed significantly reduced levels of TGF-β1 and phospho-Smad2 during early anagen and the onset of catagen development compared to WT controls.

Conclusions: Loss of integrin αvβ6 causes enhanced keratinocyte proliferation and retarded hair follicle regression in vivo, which is associated with reduced TGF-β1 expression and activation in β6−/− follicles. This study suggests that suppressing αvβ6 integrin expression levels may have a clinical application in the treatment of human hair growth disorders.

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Abstract #57: p38 MAPK Suppresses E-Cadherin Expression Through Snail Nuclear Transport During Murine Palatal Fusion

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Objectives: The TGF-β3/Smad2 signalling pathway is critical in the regulation of medial edge epithelium (MEE) transformation during palatal fusion. Previously we reported that Smad-independent TGF-β signalling through p38 MAPK may play an important role in the regulation of MEE transformation. In the present study, we examined the role of TGF-β3 regulated p38 MAPK on Snail nuclear transport and E-cadherin expression in the MEE during palatal fusion. Our objective was to examine the effect of p38 MAPK on the expression of E-cadherin in the MEE during murine palatal fusion.

Methods: In vivo – The embryonic heads (wild-type and TGF-β3-/-) at E14.25 were fixed in 4% paraformaldehyde-PBS and embedded in paraffin. Immunohistochemistry was performed with antibodies against Snail and E-cadherin. In vitro – Palatal shelves were dissected at E13 and cultured with 10 µM of SB203580, a p38 MAPK inhibitor, or 50 ng/ml TGF-β3 for 24h or 48h, and processed for immunohistochemistry. Sections were stained with antibodies against phosphorylated p38 MAPK, Snail, and E-cadherin. For quantitative RT-PCR, samples were embedded in OCT compound without fixation and the MEE cells were harvested using Laser Microdissection and Pressure Catapulting (LMPC). The quantitative RT-PCR was performed for E-cadherin and N-cadherin using LMPC samples.

Results: Phosphorylated p38 MAPK was expressed at high levels in the MEE in TGF-β3-treated organ culture samples. Snail translocated to the MEE nucleus in control samples in vivo and in vitro. Snail remained in the MEE cytoplasm in TGF-β3-/- samples in vivo and SB203580-treated organ culture. The mRNA expression of E-cadherin was increased in the MEE in SB203580-treated organ cultures.

Conclusions: During palatal fusion in the MEE, (1) p38 MAPK signalling is activated by TGF-β3, and (2) p38 MAPK regulates Snail, which then translocates to the nucleus, where it suppresses E-cadherin expression.

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Abstract #58: Craniofacial Defect Regeneration Using Engineered Bone Marrow Mesenchymal Stromal Cells

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Objectives: Large craniofacial bony defects remain a significant clinical and biomedical challenge. Bone marrow mesenchymal stromal cells (BM-MSCs) constitute a multi-potent population. Previously we developed a novel approach for BM-MSC expansion on 3D CultiSpher-S gelatin microcarrier beads in spin culture with preservation of their differentiation potential, reduction of apoptosis, and significantly enhanced trabecular bone formation in vivo. Here, we hypothesized that BM-MSCs expanded on microcarrier gelatin beads would respond to the orthopedic microenvironment, thus promoting craniofacial defect regeneration.

Methods: BM-MSCs isolated from Green Fluorescent Protein (GFP) transgenic rats were microcarrier gelatin bead expanded and transplanted into the critically-sized (5 mm in diameter) rat calvaria defects. Gelatin beads or defect alone served as controls. At 28 or 42 days, rats were sacrificed for microCT, histological, and immunohistochemistry examination.

Results: Histological measures demonstrated that the beads plus cells group induced greater new bone with a higher number of osteocytes compared to beads or defect alone groups. MicroCT results were consistent with histological data showing that BM-MSCs contributed in a statistically significant manner to the regeneration of new bone volume. Immunohistochemical staining identified GFP+ cells residing in new bone lacunae.

Conclusions: Without exogenous growth factors, rat BM-MSCs expanded on microcarrier gelatin beads followed by transplantation into the critically-sized rat calvaria defects were able to respond to the orthopedic microenvironment in vivo. GFP+ BM-MSCs, at least in part, contributed to calvaria bony defect regeneration and produced more and a higher quality of new bone compared to beads or defect alone control groups.

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Abstract #59: Comparison of Two Sonic Irrigation Systems for Smear Layer Removal

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Objectives: The current study compared the effectiveness of two different sonic intracanal irrigation systems, the EndoActivator and the Vibringe, for removing smear layer using 17% EDTA.

Methods: Fifty-four teeth having single root canals were selected and instrumented to apical size #30 using rotary Votex™ files and 3% NaOCl irrigation. After randomly dividing the teeth into three groups, each tooth was irrigated continuously over 30 seconds with 1 ml of 17% EDTA using one of 3 different methods: incorporating a 30 gauge side-vented needle endodontic syringe, or endodontic syringe plus either EndoActivator or Vibringe. Following this final EDTA irrigation, canals were dried using paper points. The crowns of the teeth were removed before splitting each root bucco-lingually into 2 halves for SEM analysis. Smear layer removal in the apical third was evaluated on separate 5-point scales at 200X magnification. Statistical analyses were performed using the Kruskal-Wallis test at the p<0.05 level of significance.

Results: Although the Vibringe and EndoActivator showed more smear layer removal than traditional EDTA syringe delivery, there was no statistical significance amongst the three groups.

Conclusions: Irrigation delivery/sonic activation for 30 seconds demonstrated that the EndoActivator and the Vibringe were equally effective in smear layer removal in the apical third of the root canal. EDTA delivery via an endodontic syringe alone is as proficient as the two sonic irrigation systems for smear layer removal in the apical third.

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Abstract #60: Sleep Disordered Breathing in Edentulous Subjects

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Objectives: The aims of this study were to assess the prevalence of suspected Sleep Disordered Breathing (SDB) in edentulous subjects and to evaluate the relationship between SDB symptoms and denture use during sleep by a questionnaire survey.

Methods: The subjects were the edentulous participants in the UBC undergraduate clinic who were scheduled for upper and lower denture fabrication. At the commencement of treatment, all subjects were asked to complete a SDB questionnaire which include questions about their denture use during sleep and the Berline questionnaire. Based on the Berline questionnaire, subjects were divided into two groups (high and low risk). Statistical analysis was performed with SPSS 10.0 software with student t and chi-square tests. A P value of less than 0.05 was considered significant.

Results: A total of 62 edentulous subjects (50.0% male) completed the questionnaire. Age and Body Mass Index (BMI) were 70.7±10.3 years and 27.7±5.7, respectively. Some 25 patients (40.3%) wore the denture during sleep, 16 subjects (25.8%) wore both the upper and lower dentures, and 9 subjects (14.5%) wore only the upper denture. Some 37 patients (59.7%) slept without their dentures. The questionnaire suggested that 40.3% of the participants in the UBC denture module had an increased chance of exhibiting SDB. Eleven subjects (29.7%) in the low risk group used their denture during sleep while 5 patients (20.0%) in high risk group did. There was no significant difference between the groups in terms of age, BMI, or denture use during sleep.

Conclusions: This study suggests that the prevalence of suspected SDB in edentulous subjects is 40.3%, which is higher than the general population (Young et al., 1993). Dentist should consider edentulous patients as having a high risk of SDB. Denture use or lack thereof did not appear to affect the risk of SDB in this study.