THE UNIVERSITY OF BRITISH COLUMBIA

Faculty of Dentistry

RESEARCH POSTER ABSTRACTS

RESEARCH DAY 2019

1. Association of Adverse Events to the Efficacy of Optically-Guided Surgery

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Objectives: Despite many advances in cancer treatment, oral cancer remains a major health problem worldwide due to its aggressive nature. Recognizing this, traditional surgical resectioning with white-light (WL) involves a significant margin of surrounding normal-looking oral mucosa. Still, the unpredictability of the occult disease allows extension beyond tumour clearance area. This incomplete recognition of the field extension can result in local recurrences (LR) at the primary site. The newly-developed approach using fluorescence visualization (FV) has demonstrated improved surgical tumour margin decision. The objective of the study was to determine the efficacy of this tool in relation to adverse events (AE).

Methods: The Canadian Optically-guided approach for Oral Lesions Surgical (COOLS) trial has recruited 400 patients with severe oral dysplasia or carcinoma *in situ* (n=160) and invasive squamous cell carcinoma (n=240) from nine centres. Out of 400 patients, data for 124 patients from British Columbia were first evaluated. A total of 11 patients were excluded due to lack of follow-up information. Data on LR and AE were collected in FV- and WL- guided groups. AEs were self-reported by patients and categorized based on the National Institute of Health/National Cancer Institute Common Terminology Criteria for AEs, 1 being mild and 5 being death-related.

Results: The FV-guided (n=56) and WL-guided groups (n=57) each had 13 patients with LR (23%). There was no major difference in the number of AEs between FV and WL- guided groups (217 vs 208). Most AEs for all groups were oral pain, oral swelling, oral dysesthesia, and fatigue.

Conclusions: Within a controlled trial setting, our preliminary data showed no significant differences in LR between FV- and WL- guided groups. FV-guided surgery showed similar AEs compared to the WL group, i.e., no further harm to the patients using this new approach. We plan to complete the entire set of 254 patients to confirm this observation.

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2. Elder Abuse and Neglect: Undergraduate Dental Students' Knowledge and Pedagogies

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Objectives: The objectives of this study were to explore undergraduate dental students' knowledge and attitudes about elder abuse and neglect, and to review the suggested pedagogies to teach these topics in dental education.

Methods: An anonymous 11-item questionnaire was provided to a total of 311 undergraduate second-year dental students at the UBC Faculty of Dentistry from 2011 to 2018. The questions covered types of abuse, reporting mechanisms, and implications of elder abuse and neglect, with the answers in a true or false format. Data were analyzed by questions' content using descriptive statistics, while an ANOVA test was used to compare variation in the responses (p-value = 0.05) using SPSS 21. A semi-systematic review on elder abuse and neglect using the keywords 'dental education' AND 'elder abuse' AND 'pedagogy' was carried out via PubMed/MEDLINE during the summer of 2018.

Results: The questionnaire was completed by 213 (68%) students. None of the students answered all the questions correctly, while 46 (21.5%) got 3 or less questions wrong. The majority of students (73.7%; 157) were aware that abuse and neglect constitute human rights violations, but only 8.2% (19) knew about the existence of resources for reporting and support. There was no statistically significant difference in the rate of correct responses between the academic year. One peer-reviewed publication was found that presented the teaching pedagogies used to discuss elder abuse and neglect in dental education, and involved mostly didactic lectures and case-based discussions.

Conclusions: Elder abuse and neglect occur with a high frequency and can display intra- and extra-oral signs and symptoms visible to a dental professional. It remains important to address such issues within undergraduate dental education to raise awareness. The teaching of reducing elder abuse and neglect should go beyond awareness and should employ engaging pedagogies beyond lectures.

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3. Children's Health and Oral Health Services in Northern British Columbia

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Objectives: Children living in northern British Columbia (BC) are more likely to have poorer oral health than children living in urban areas. This is partly attributable to the limited availability of oral health services. The region's extent of available private and public oral health services is not well quantified. The aim of the project was to identify, describe, and map the current availability of health and oral health services for children residing in northern BC.

Methods: An electronic search identified the distribution and location of northern population centres and First Nations (FN) communities. Further electronic searches established the locations of private dental clinics, public dental program health units, public medical health units, public primary care medical clinics, and private medical clinics. E-mail and telephone correspondence with medical and dental providers confirmed the types of oral health service provision. All data was inputted into ArcGIS software to map the distribution of population centres, dental services, and physician services.

Results: Northern BC contains 21 population centres and 10 rural areas. There are 128 dentists, 185 dental hygienists, and 67 private dental offices in 16 population centres and 6 rural areas. There are 450 general family physicians in 159 locations in 21 population centres, and 9 rural areas. Public dental programs are available in 7 population centres. There are 54 FN communities in northern BC, of which 18 receive the Children's Oral Health Initiative program. Sixteen FN communities have the Dental Therapy program, with 8 communities receiving the full scope of services, 1 receiving limited services, and 7 with vacant provider positions.

Conclusions: More than twice as many physicians practice in northern BC compared to dentists. Therefore, collaboration between dentists and physicians to promote preventive dental care delivery by physicians may help address the persistent oral health disparities in regions where dental services are limited.

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

4. Radiation Therapy's Impact on Salivary Amounts, pH and Mouth Opening

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Introduction: Cancer treatment has been shown to cause multiple side effects, including xerostomia and trismus, due to salivary gland and muscle damage during radiotherapy. However, the use of intensity modulated radiation therapy (IMRT) to replace more invasive conventional beam radiation therapy means that more healthy tissue sparing can now be achieved. No study has been published on IMRT's effect on salivary pH and mouth opening. The aim of the study was to determine the impact of IMRT on salivary rates, pH, and mouth opening.

Methods: Thirty-six patients diagnosed with cancer had their whole resting saliva, stimulated saliva, respective pH, and maximum mouth opening at baseline documented, upon first being seen at the BC Cancer Agency prior to receiving IMRT, and subsequently 3 months post IMRT. Pre- and post-treatment salivary volume, pH, and mouth opening were compared using paired t-tests.

Results: Resting saliva was significantly reduced post-treatment, $t(35) = 3.994$, $p < 0.0005$. Stimulated saliva was also significantly reduced, $t(33) = 4.980$, $p < 0.0005$. There was no significant difference in resting or stimulated saliva pH before and after treatment, $t(26) = 1.616$, $p = 0.118$, and $t(30) = 1.239$, $p = 0.225$, respectively. Mouth opening distance was significantly lower after treatment, $t(30) = 3.058$, $p = 0.005$.

Conclusions: IMRT can cause a statistically significant decrease in stimulated saliva volume, although no significant decrease was observed in resting salivary volume. Resting and stimulated salivary pH did not appear to have been impacted by IMRT. At the 3-month mark, a statistically significant decrease in maximum mouth opening can already be observed. Although limited in sample size, this study does highlight the need for early trismus therapy on cancer patients, and encourages us to further study the impact of IMRT on xerostomia and oral health in order to propose better strategies for treatment.

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5. The State-of-the-Art in Teaching Caries Control Agents

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Objectives: To investigate the teaching practices and perceived barriers to discussing caries control agents in Canadian dental and dental hygiene schools and to review the available pedagogies involved in teaching such topics at undergraduate levels.

Methods: A brief 10-item questionnaire was distributed to all 10 dental schools and all 32 dental hygiene programs in Canada over the summer of 2018. The questions covered the types of caries control agents taught and the pedagogies used to educate students on those agents. A semi-systematic literature review was conducted using the keywords "caries control agents" AND "silver diamine fluoride (SDF)" AND "education", on PubMED/Medline®. Two independent pairs of researchers scrutinized the publications at title, abstract, and full-text levels until consensus. Included publications were limited to English, Portuguese, and Spanish languages.

Results: Seven dental schools and 23 dental hygiene programs responded to the questionnaire; of those, 93% discussed SDF mostly via lectures, while 30% used SDF clinically (all in primary teeth). Approximately half of the programs/schools (n = 14) mentioned unclear guidelines for clinical use of SDF as a barrier to teaching and implementation. The literature review yielded 307 hits, of which 26 full-text articles were included. Twelve studies discussed the teaching of caries control with didactic lectures as the sole methodology and one study discussed the teaching of SDF at graduate level only.

Conclusions: Although all schools and programs teach caries control in general, not all of them include SDF - with unclear guidelines cited as the main barrier preventing the teaching and use of SDF clinically. Most of the teaching pedagogies used to present caries control agents have been in the form of didactic lectures. As the use of SDF becomes more popular, follow-up studies should explore the extent to which dental professionals are utilizing SDF in their practices.

6. Participatory Design Improves Oral Self-Care in High Caries-Risk Children

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Objectives: To use outcomes from a needs assessment to design and implement sustainable, community-driven interventions that reduce risk factors associated with dental caries in a rural Himalayan boarding school.

Methods: In 2017, 486 students from kindergarten (KG) to grade 10 were examined during dental screenings for number of cavitated lesions and surveyed about dental pain and oral self-care behaviours. An evaluation of oral self-care perspectives and practices was assessed through focus interviews and observations of students, workers, and community members. The collective information was shared with school personnel and students to facilitate a participatory discussion around designing and implementing interventions to improve oral self-care among students. In 2018, the interventions were assessed for sustainability and their impact on changing oral health perspectives.

Results: In 2017, 62% of children presented with at least one carious lesion. Those students that had four or more carious lesions were mostly from younger age groups. Members of the school and community reported a high consumption of cariogenic foods, lack of oral self-care routines, and the loss of toothbrushes as contributing factors to high caries prevalence. Five interventions were designed and implemented that focused on reducing the identified risk factors. In 2018, almost 15% fewer students identified as not having a toothbrush, with the greatest improvement occurring in the younger age groups. Most significantly, there was a 46% increase in the number of students brushing once a day. Most students agreed that the interventions implemented the year before made them more aware of the importance of oral health.

Conclusions: A participatory design approach in the Spiti Valley has been effective in creating sustainable behavioural changes over a short period of time. Such methods can be effective in reducing the risk factors associated with dental caries and reduce the burden of dental disease in rural populations.

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7. Medication-Related Osteonecrosis of the Jaw in BC: a Prospective Study

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Objectives: MRONJ is defined as maxillofacial bone exposure, in patients currently/previously on antiresorptive/antiangiogenic agents (largely bisphosphonates/denosumab). Recently bisphosphonates/denosumab have been widely used in patients with severe osteoporosis, multiple myeloma, or metastatic bone cancers. The objective of the study was to explore the incidence and risk factors of MRONJ in British Columbia, which currently are not clear.

Methods: We began recruiting patients from BC Cancer who were, or would be, on bisphosphonates/denosumab. The patients were initially interviewed with structured questionnaires to collect demographics, medication history, and other risk factors (social history, dental and medical conditions). They were scheduled for a follow-up either every 3 months (if they had recent extractions) or at 6 months. At follow-up, patients were interviewed using structured questionnaires to record any changes to their medications or other history, then screened by the oral surgeon for ONJ. Lesions were documented by structured forms and photographs. Follow-ups are planned for the 3-year mark.

Results: From June 21 to November 1, 2018, 50 patients were recruited to the prospective arm. Thirty-nine (78%) were female and 11 (22%) male. Forty-eight had cancer, with breast (54%) and multiple myeloma (23%) as the most prevalent. Zoledronate (N=24), Pamidronate (N=17), and Denosumab (N=5) were administered most commonly. In this period, 3 patients, who were all on denosumab for more than 1 year (1, 1.5, and 3 years), developed MRONJ. Two previously used alendronate (one for 3 years, the other 8 years). One had recent extractions (1 month prior to lesion development).

Conclusions: Although it is the beginning of the study, we have already found a sizeable number of patients on high dose bisphosphonates/denosumab. With more sample and lesion development in the future, we will further determine the incidence and risk factors involved in MRONJ, with a hope to understand the benefit and harm of using BP/denosumab in cancer treatment.

8. 3D Analysis of Viscerocranial Asymmetry During Human Fetal Development

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Objectives: Asymmetry has been noted in the human craniofacial region in several pathological conditions and growth abnormalities, often with a directional predilection. Physiological asymmetry has also been reported in normal adults and adolescents, with certain regions of the cranioskeleton, such as the mandible, displaying prevalent asymmetry. However, the existence and extent of asymmetry in the craniofacial regions during fetal development have not been evaluated. The objective of this study was to assess the degree of asymmetry in facial bones during the fetal stages of human development.

Methods: Twenty-one preserved conceptuses from the Congenial Anomaly Research Center at Kyoto University, between the ages of 15-20 weeks gestation, were studied using high resolution micro-computed tomography (μCT) imaging. Digitally segmented facial bone pairs were annotated with landmarks, in duplicate, and the coordinates were tested for reliability. Traditional methods of asymmetry analysis rely on manual establishment of midsagittal planes as a reference. However, this was not possible in our dataset due to lack of midline ossification. Hence, we analyzed asymmetry using geometric morphometric (GM) approaches as well as adapted deformation-based asymmetry (DBA) methods.

Results: We obtained good reliability for landmark placement. Morphometric analysis revealed that the developing facial bones display statistically significant directional and fluctuating asymmetry ($p=0.0109$ and $p<0.001$, respectively). DBA methods suggest that the magnitude of asymmetry in the facial bones is low and does not appear to be correlated to the estimate of overall size of conceptus ($R=0.51$). Additionally, the patterns of asymmetry are highly variable between individual specimens.

Conclusions: The developing fetal facial skeleton displays variable patterns of low magnitude asymmetry. GM and DBA methods offer unique advantages to quantitative and qualitative assessment of facial asymmetry. Together, these specimens are providing comprehensive information about prenatal morphogenesis of the human craniofacial region.

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9. CSF-1 Receptor Inhibition Results in Mouse Incisor Malformations

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Objectives: Colony-stimulating factor-1 receptor (CSF-1R) plays a key role in the regulation of mononuclear phagocyte development, osteoclastogenesis, bone formation, and neuronal differentiation and survival. A recent study from our group found that inhibition of CSF-1R using a pharmacological model in utero, resulted in significant defects in dental morphology postnatally. The aim of the study was to characterize these defects using histology.

Methods: The CSF-1R inhibitor PLX5622 or vehicle control was administered through diet to pregnant mice from embryonic day 3.5 to birth (e3.5 – P0). Heads of offspring were collected at postnatal day 21 (P21) for histological analysis. Samples were decalcified first with 14% EDTA, then with 5% Gooding solution. Samples then underwent tissue processing and embedding in paraffin wax. Tissues were sectioned at 5 μm thickness and stained with Hematoxylin and Eosin.

Results: Abnormalities were observed in the incisors of P21 PLX5622 diet-exposed mice, with variability in presentation. The maxillary incisors appeared branched or geminated, showing a flipped, duplicated tooth structure fused to the original incisor, both sharing the same dental follicle. The mandibular incisors revealed irregular infoldings of the dentin-enamel junction with grossly disorganized dentin and pulp, giving the appearance of multiple small internal "germinating centers". Surprisingly, the ameloblasts and odontoblasts maintained their normal columnar shape and polarity, except for regions of infoldings, where the cells are flattened with unorganized regions of mineralization. The results obtained histologically match those obtained from micro-computed tomography (μCT) scanning.

Conclusions: Histological analysis provides evidence for the role of CSF-1R during odontogenesis. Furthermore, these results suggest that signalling through CSF-1F is likely important during the initiation and morphogenesis stages when the shape of the enamel organ and dentino-enamel junction is being established. CSF-1R is possibly less important during the histodifferentiation and mineralization stages.

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10. Platelet Shape Change Induced by Lipopolysaccharide (LPS) from *Porphyromonas gingivalis*

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Objectives: Periodontal disease (periodontitis) is associated with a higher risk of blood clot formation and thrombosis. Lipolysaccharide (LPS) is the antigenic cell wall component of *Porphyromonas gingivalis,* a pathogen central to the etiology of periodontitis. The interaction of LPS and platelets is thought to be mediated by Toll-like receptor 4 (TLR4), which platelets are known to express. We therefore hypothesized that exposure to LPS may accelerate the shape change in platelets that is required for clot formation.

Methods: Blood samples were obtained from healthy human volunteers. Platelets were isolated from blood via centrifugation and washed by resuspension in a washing buffer. Platelets were allowed to spread on glass coverslips in the presence or absence of *P. gingivalis* LPS for time periods ranging from 1-15 minutes. Platelets were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton-X, and stained with Alexa-488-phalloidin to visualize F-actin (polymerized actin) via confocal microscopy. In some experiments, platelets were pre-treated with ML141, an inhibitor of the small GTPase Cdc42, which is responsible for the formation of filopodia (spikes) characteristic of spreading platelets. The experiment was performed 3 times (independent replicates), and 10 pictures were taken of each treatment group at each time interval. The number of filopodial extensions were counted for each cell and averaged to provide a measure of platelet spreading for each treatment group.

Results: *P. gingivalis* LPS did induce a significant ($p<0.05$) increase in platelet spreading and filopodial formation. Pre-treatment of platelets with the inhibitor ML141, however, did not significantly inhibit spreading or filopodial formation at the tested concentration (nanomolar range).

Conclusions: *P. gingivalis* LPS stimulates filopodial formation and cell spreading in platelets. In future experiments, ML141 (and/or other Cdc42 inhibitors) should be tested at different concentrations to better define the LPS-Cdc42 signalling pathway.

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11. Assessing Oral Cancer Field Intralesional Heterogeneity Using Quantitative Tissue Phenotype

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Objectives: Oral cancer (OC) is known to develop from a heterogenous field containing genetically altered cells with various malignant formation rates, defined as field cancerization. Histology alone is unable to detect these subtle phenotypic changes. This study aimed to use quantitative tissue pathology (QTP) for calculating nuclear phenotype scores (NPS) on surgical samples. We hypothesized that NPS can provide an objective approach to assess OC fields for intralesional heterogeneity and recurrence.

Methods: A case study was planned on 30 OC samples previously collected in a Pan-Canadian surgical trial. For each case, two consecutive 5 µm sections were used, one stained with hematoxylin and eosin, and one with Feulgen-thionin for QTP analysis. Scanned slides were reviewed and demarcated into regions of interest (ROIs) by the pathology: low-grade lesion (LGL, hyperplasia to mild dysplasia), moderate dysplasia (D2), high-grade lesion (HGL, severe dysplasia to carcinoma). Within each ROI, nuclei were analyzed for 96 QTP features relating to nuclear morphology and chromatin texture to create a final NPS score.

Results: QTP analysis was completed for 11 tissue samples with 124 ROIs and 26,854 nuclei in total. A significant increase of NPS was observed, going from LGL, D2, to HGL (0.25±0.21, 0.47±0.34, 0.55±0.32, $p<0.0001$); however, there was no difference between D2 and HGL. Two cases showed low NPS (0.13±0.040) and one case showed high NPS (0.92±0.03) across the field, even with varying degrees of pathology. Two cases showed heterogeneity (multiple high peaks) within the fields, with the highest NPS at D2 regions. Interestingly, four LGL margins showed high NPS (>0.55).

Conclusions: Our study demonstrated that QTP analysis provides an objective approach to investigate tumor heterogeneity among histologically similar areas within an OC field. We plan to complete 30 samples to further investigate identifiable phenotypes at the tumor marginal tissue that are associated with disease recurrence.

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12. Manufacturing Doxycycline and Metal Ion Loaded Polymethylmethacrylate Microspheres

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Objectives: Polymethylmethacrylate (PMMA) has been considered a biocompatible material for use in dentistry and medicine. Production of PMMA microspheres loaded with drugs can be valuable when incorporated into orthopedic and dental cements. The objectives of this study were to test the feasibility of incorporating doxycycline and metal ions into PMMA microspheres (50 µm diameter), to evaluate the drug loading efficiency, and the drug release profiles.

Methods: Four groups were tested: 1) Pure PMMA-microspheres (25% weight), 2) PMMA-microspheres containing 15% doxycycline, 3) PMMA-containing 5% metal salt, and 4) PMMA-microspheres containing 5% metal salt and 15% doxycycline. Doxycycline and metal salt loading and drug release profiles were analyzed using HPLC and ICP-MS, respectively. All microspheres were observed under a light microscope at 100× magnification during manufacturing for spherical shape, drug encapsulation and a targeted 50 µm diameter. The formulation containing 5% metal salt + 15 % doxycycline was analyzed in a Malvern Mastersizer instrument.

Results: The lead formulation of PMMA containing theoretically maximal amounts of 5% metal salt + 15% doxycycline showed 8.3 ± 0.64% doxycycline drug loading to give a 55% encapsulation efficiency, along with 0.36 ± 0.03% metal salt loading for a 7.2% encapsulation efficiency. Similarly, PMMA/15% doxycycline alone showed 8.1 ± 0.88% doxycycline drug loading and 54% encapsulation efficiency. PMMA/5% metal salt alone presented 0.46 ± 0.07% drug loading and 9.3% encapsulation efficiency. The lead formulation (PMMA/15% dox/5% metal) showed continued metal salt and doxycycline release for up to 15 days in biphasic media. The method used successfully produced microspheres with a median diameter close to 50 µm.

Conclusion: PMMA microspheres containing both doxycycline and metal salt were successfully manufactured with a target diameter of 50 µm. The final encapsulation efficiencies for the two drugs (approximately 55% doxycycline and 7% metal salt) provided suitable microspheres for incorporation into orthopaedic and dental materials.

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

13. EGFR Inhibitors Prevent αvβ6 Integrin Downregulation in Gingival Epithelial Cells

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Objectives: In periodontal diseases, multispecies bacterial biofilms accumulate between the epithelium of gingiva and teeth resulting in inflammation, periodontal pocket formation, and alveolar bone loss. Integrin αvβ6 maintains anti-inflammatory transforming growth factor-β1 (TGF-β1) signaling in healthy junctional epithelium. However, it is significantly reduced in the pocket epithelium in periodontal disease. We have shown previously that β6 integrin mRNA and protein expression is suppressed by bacterial biofilms in cultured gingival epithelial cells (GECs). Recently, we found that biofilm-induced suppression of β6 integrin expression was driven by autocrine epidermal growth factor receptor (EGFR) signaling. Therefore, we hypothesized that blocking the EGFR activation prevents β6 integrin downregulation in GECs.

Methods: GECs were exposed to oral biofilm extract or EGFR ligands, and the expression of β6 integrin was analyzed by RT-qPCR. Chemical inhibitors (AG1478, Afatinib, Canertinib, Dacomtinib, Erlotinib, Gefitinib, Lapatinib) were used for blocking EGFR signaling in GECs. Integrin β6 expression level was analyzed by In-Cell Western blotting. FVB mice were silk-ligatured to induce experimental periodontitis in the presence or absence of EGFR inhibitors, either locally or systemically, for 2 weeks. Mice were then sacrificed, and the bone loss and inflammation levels in the mouse jaws were analyzed by micro-CT and histology.

Results: Oral biofilm extract and EGFR ligands reduced β6 integrin expression by 50% in GECs. EGFR inhibitors prevented EGFR-ligand-induced β6 integrin downregulation in GECs and significantly reduced bone loss and inflammation in the mouse periodontitis model.

Conclusions: The biofilm-initiated β6 integrin downregulation in GEC can be prevented by blocking EGFR signaling. In addition, selective EGFR inhibitors significantly reduce periodontal inflammation and bone loss in an experimental periodontitis model *in vivo*. Therefore, blocking EGFR signaling could serve as a novel approach to reduce inflammation and bone loss in periodontal disease.

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14. Osteogenic Differentiation of Human Gingival Fibroblasts

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Objectives: Periodontitis results in bone loss and eventual tooth loss. The current available options for bone regeneration include guided tissue regeneration and the use of bioactive agents. These methods can effectively regenerate bone in small periodontal defects. However, larger periodontal defects are still challenging to treat. Hence, the use of human gingival fibroblasts (HGFs) as a cell-based therapy has been suggested as an alternative treatment option. HGFs are readily available with minimal donor site morbidity and may be ideal for tissue engineering efforts in regenerating lost alveolar bone. We hypothesized that osteogenic induction of HGFs would promote bone regeneration. Therefore, the aim of this study was to investigate the extent of osteogenic differentiation of HGFs *in vitro*. Our long-term aim is to determine the osteogenic potential for HGFs to develop a cell-based therapy for bone regeneration in periodontitis patients.

Methods: HGF strains isolated from six different donors were exposed to osteogenic medium for 4 weeks (basic medium, 50 mg/mL of ascorbic acid, 100 nM dexamethasone, 100 nM vitamin D3, and 10 mM beta-glycerophosphate). Osteogenesis was evaluated based on Von Kossa staining, scanning electron microscopy (SEM), and energy-dispersive X-ray (EDX) after 4 weeks.

Results: Based on Von Koss staining, cultures were stratified into categories based on the magnitude of calcification: no calcification (0%), poor calcification (<10%), moderate calcification (10-40%), and significant calcification (>50%). There was no calcification in the controls cultured in non-osteogenic medium. Four cell strains showed significant calcification, one moderate calcification, and one showed no calcification with osteogenic treatment. Calcified cultures showed diffuse or patterned calcifications. SEM images and EDX mineral distribution confirmed the presence of calcium (Ca) and phosphate (P) in the calcifying nodules with Ca/P ratio ranging between 2 and 2.5.

Conclusion: Under appropriate osteogenic conditioning, HGFs may provide a source for cell-based therapy for bone regeneration in periodontitis patients.

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15. Regulation of Profibrotic TGF-β Signalling by CD26 in Skin Fibroblasts

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Objectives: A skin fibroblast (SFBLs) population which expresses high levels of CD26, a multifunctional cell surface protein that regulates ATP-signalling, may drive skin fibrosis and scarring. In the gingiva where wound healing results in minimal scarring, this distinct population of $CD26^+$ fibroblasts is largely absent. We have shown that downregulation of CD26 in SFBLs by siRNA modulates expression of profibrotic TGF-β signalling pathway target genes. Thus, we hypothesized that downregulation of CD26 regulates the profibrotic phenotype of SFBLs by ATP and TGF-β signalling. Our objective was to elucidate how CD26 regulates this pathway.

Methods: Human SFBLs were transfected with control or CD26 siRNA in three-dimensional *in vivo*-like cultures. Cells were serum-starved and cultured for 24 h before treatment with, or without, pharmacological inhibitors targeting profibrotic signalling pathways or functions of CD26. Gene and protein expression were analyzed by real-time RT-PCR and Western blotting, respectively.

Results: CD26 siRNA treatments led to marked suppression of CD26 mRNA (>90%) and protein levels (>70%). Downregulation of CD26 resulted in concomitant phosphorylation of SMAD3, a key effector of TGF-β signalling, at both S423/425 activation and S208 inhibitory sites, and upregulation of TGIF, a transcriptional repressor of TGF-β signalling. Pharmacological inhibition of neither CD26 catalytic functions nor a set of molecules involved in ATP-adenosine signalling resulted in modulation of gene expression or TGF-β signalling in SFBLs. However, CD26 siRNA treatments led to significant upregulation of mRNA and protein levels of Cx43, a cell surface molecule that regulates cell-to-cell communication, ATP release and intracellular TGF-β signalling.

Conclusions: Downregulation of CD26 involves upregulation of Cx43, paracrine TGF-β induced phosphorylation of SMAD3 at activating S423/425 and inhibitory S208 sites, and an increase in TGF-β1 signalling repressor TGIF in SFBLs. This pathway may provide a novel target to modulate the profibrotic phenotype of SFBLs.

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16. Landmark-Based 3D Morphometrics of Dental Crypts During the Middle Trimester

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Objectives: In this study we describe growth characteristics of the teeth and jaws in a collection of fetal specimens housed at the UBC Faculty of Dentistry. Teeth pass through bud, cap, and bell stages in the middle trimester. As the teeth develop, the intramembranous bone begins to be deposited. It is not clear whether the bone is instructive or merely supportive of tooth development. We tested the hypothesis by analyzing the shapes of the mandibular crypts, in 3D, over the middle trimester.

Methods: Micro-CT scans of human fetal heads (n= 25, 12-19 weeks gestation, 3-4 specimens per time point) were used. Mandibular growth was analyzed by segmenting out the mandible and measuring bone volume (Amira software). Thirty-nine landmarks were chosen on each hemi-mandible in reproducible areas of the mandibular crypts. Fifty teeth for each morphotype were studied. Intra-observer error was calculated by placing landmarks on the same mandible 1 week apart. Geometric Morphometric analyses (e.g. Procrustes superimposition, principal component, canonical variate, and discriminant function analysis) were performed using MorphoJ software.

Results: The principal component and canonical variate analysis revealed that there are significant shape differences in the crypts between 12-14 weeks and 17-19 weeks. The intermediate period 15-16 weeks was not significantly different than the early or later timeframes. These trends matched segmented volumetric data for the mandible. Shifts in specific landmarks, particularly the buccal landmarks adjacent to m1 and c, were observed between the early and late period. Allometric growth in the gingival direction was also observed for all tooth crypts.

Conclusions: Our data suggests that all the tooth germs are interacting with the bone that will later surround the roots. The internal surfaces of the crypts appear to be displaced apically and buccally, consistent with bone remodelling that has been reported by others.

Acknowledgements: Supported by a UBC Faculty of Dentistry Student Summer Research Award to DP. Operating funds are from the Faculty of Dentistry. Human ethics approval number: H08-02576.

17. Modulation of Skin Wound Healing by Bioengineered Gingival Fibroblast Grafts

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Objectives: Gingival wounds heal faster and form less of a scar than skin wounds. This may depend on phenotypic differences between gingival (GFBLs) and skin fibroblasts (SFBLs). We hypothesized that GFBLs may be used to mitigate scar formation in skin wounds. This study aims to develop a preclinical red Duroc pig model to test whether tissue-engineered autologous GFBLs containing grafts suppress hypertrophic scar formation compared to SFBLs. Healing of deep wounds in these animals results in hypertrophic-like scar formation similar to humans.

Methods: Autologous GFBLs and SFBLs were isolated from red Duroc pigs and seeded on resorbable polyglactin woven mesh (Vicryl) constructs. The bioengineered constructs were placed in standardized shallow (0.8 mm; do not form scars) and deep (2.4 mm; form hypertrophic-like scars) wounds created in the dorsal skin of the pigs and wound healing was followed clinically by standardized photographs and by histological and immunohistological analysis up to 92 days post-surgery.

Results: DiOC-labelled autologous GFBLs and SFBLs were detected in the wounds up to 92 days after wounding. GFBL-treated shallow wounds showed faster re-epithelialization compared to SFBL-treated and control wounds (Vicryl mesh-only treated or non-treated wounds). Connective tissue of the GFBL-treated wounds showed faster extracellular matrix maturation and abundant accumulation of tenascin-C, typical to gingival wounds. GFBL-treated deep wounds showed faster and greater pigmentation and reduced wound contraction/distortion compared to the other treatment groups. Treatment of both shallow and deep dermal wounds with GFBLs appeared to suppress the abundance of profibrotic CD26-positive fibroblasts in the tissue.

Conclusions: Autologous GFBL-containing constructs can deliver viable cells into skin wounds where they are maintained for at least 92 days and may promote fast wound healing and suppress scar formation. The approach shows promise to further study the utility of GFBLs to alleviate pathological scar formation in skin.

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18. Optimizing Quantum Dot Immunolabeling on Formalin-Fixed Paraffin-Embedded Tissue

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Objectives: Quantum dots (QD), fluorescent semiconducting nanoparticles, provide many advantages over conventional fluorescent dyes, notably their narrow emission spectra and biofunctionality. The overall objective is to develop a simple, robust method to create QD-primary antibody conjugates that will allow direct immunolabeling of biomarkers on formalin-fixed, paraffin-embedded (FFPE) tissue.

Methods: We have developed a step-by-step protocol using QD-protein (SpA)-primary antibody (Ab) to stain FFPE sections. Five different water soluble QDs (carboxybetaine, sulfobetaine, polyethylene glycol, glutathione, and dextran) were tested for their degree of non-specific binding to FFPE tissue. Functionality tests, Dynamic Light Scattering (DLS), and Nanoparticle Tracking Analysis (NTA) were performed to confirm formation of QD-SpA-Ab scaffolds. We also tested a novel tetrameric antibody complex (TAC) coupled with an Ab of interest (Ki-67, a mouse IgG1) that either directly bound to QD or indirectly to QD after TAC incubation with FFPE tissue. Additionally, to maximize the surface area of cells, we plan to test the protocol on cell suspensions using dissociated cells from FFPE tissue with both direct and indirect approaches. Negative controls without Ab and positive controls using conventional immunohistochemistry were also performed. Fluorescence was captured using a fluorescence microscope and a camera. Images were analyzed with ImageJ.

Results: QD-carboxybetaine showed the least amount of non-specific binding and was used for all subsequent QD-SpA immunolabeling experiments. DLS and NTA have confirmed the formation of QD-SpA-Ab scaffolds through observation of an increase in scaffold size with each additional protein added (SpA added to QD then antibody added to QD-SpA). Direct immunolabeling with QD-TAC showed the most specific binding, compared to direct QD-SpA and indirect QD-TAC approaches. Testing on cell suspensions using various approaches requires continued optimization.

Conclusions: A new technique has been established to perform QD immunolabeling of FFPE tissues. Further refinement of the protocol is required.

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19. Long-Term Dimensional Changes Associated with Slow Maxillary Expansion

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Objectives: To compare the long-term palatal symmetry, dimensions, and molar angulation changes following early mixed-dentition slow maxillary expansion with untreated controls.

Methods: A total of 30 patients treated with a Haas-type expander for unilateral posterior crossbite with functional shift were compared with 30 controls matched for dental age, gender, and molar relationship. Records were taken before (T1), after expansion (T2) and 4 years after expansion (T3). Palatal width, surface area, volume, and molar angulations were measured on digitized models. Surface area and volume were split in half then divided into anterior, middle, and posterior segments to measure symmetry.

Results: T1-T2: Mean intercanine width increased 4.7 mm and intermolar width increased 4.8 mm. The treated mean surface area increased 130.6 mm^2 compared with 14 mm^2 in the controls. The treated palatal volume increased 946.4 mm^3 compared with 200 mm^3 for the controls. T2-T3: Mean intercanine width relapsed 3 mm and intermolar width relapsed 1.1 mm. At T3, there was no significant difference between the treated sample and the controls for the intercanine width, total surface area, and total volume. After expansion, the first permanent molars showed an increased buccal and distal inclination after treatment, opposite to the controls. The increase in buccal inclination was greater on the crossbite side. After relapse, the molars became more upright than the originals on both sides.

Conclusions: Slow maxillary expansion with a Haas-type appliance results in similar expansion across the canines and first molars. A palate that is symmetrical before expansion may become asymmetric immediately after expansion, but only in the middle and posterior segments. After long-term follow-up, no significant difference was observed between the treated sample and controls for intercanine width, total palatal volume, and surface area. Changes in individual molar angulations following palatal expansion can be evaluated without radiation imaging.

20. Authoritative Parenting Model for Improving Oral Hygiene in Orthodontic Patients

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Objectives: The objective of this prospective randomized clinical study was to assess the effectiveness of an authoritative parenting model in improving the oral hygiene skill level of adolescent orthodontic patients.

Methods: The sample consisted of patients aged 10-16 years undergoing orthodontic treatment at UBC's graduate orthodontic clinic. Patients were randomized into two study groups: an intervention group receiving oral health promotion material and a template of a parent-child contract, and a control group that received conventional dental instructions provided by orthodontic graduate students. Oral hygiene skill levels were assessed by measuring the percentage of total plaque (after brushing) at three observation periods. Plaque scores were calculated from the photographs of teeth with disclosed plaque, employing the manual for standardized digital estimation of dental plaque scores.

Results: Overall, patients had high plaque scores with large variations in oral self-care skills. Although skills improved significantly from the baseline in both study groups, there was no statistically significant difference ($p>0.05$) between the intervention group and the control group. The compliance rate with the intervention was low (~30%); however, within the compliant group, there was a nonsignificant trend for improvement. The baseline plaque level was the only statistically significant predictor for future improvement.

Conclusions: The authoritative parenting model did not result in greater improvements in oral self-care skills of orthodontic patients in comparison to the conventional dental instruction. Compliance with the intervention was low; therefore, it is important to identify reasons for non-compliance.

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21. Evaluating Access to Dental Care for People Living with HIV

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Objectives: It is important that people living with HIV/AIDS (PLWHA) receive routine oral health care due to their increased risk of oral complications. However, PLWHA have difficulty accessing oral health services as they continue to endure HIV-related stigma and discrimination. Since September 2011, the University of British Columbia (UBC) Dental Hygiene Degree Program (DHDP) implemented a preventive oral health services program at the Positive Living Society of British Columbia (PLSBC), a non-profit organization supporting PLWHA. This study aims to evaluate the impact this service delivery has on dental access for PLSBC members and the organization.

Methods: A retrospective chart review of 170 PLSBC members who utilized the preventive oral health services was conducted with descriptive analysis of the data. Personal interviews were conducted with 10 members and one focus group comprised of 12 staff and the administration. Audio-recordings were transcribed and coded thematically using N-Vivo® 11 software. Emerging themes were identified accordingly using an interpretative phenomenology approach with Penchansky's and Thomas' concept of access.

Results: PLSBC members liked that this dental outreach program was easily accessible, convenient, free, and safe. Due to past traumatic experiences, members seek specific traits in dental providers that make them feel comfortable and safe. Members perceived the program of value, as it provides an opportunity to educate future dental professionals about HIV sensitivity. Members had to accommodate to the environment, facility, and set-up, which differed from a private dental clinic. Referrals to dental practices further increased access to dental services for members.

Conclusions: Staff described this program as beneficial in helping members overcome HIV-related stigma when seeking oral health services, while at the same time training future dental professionals in cultural safety when working with PLWHA. Community-based services that provide ongoing preventive dental care and education may improve access to dental care for PLWHA.

Acknowledgements: This study was supported by the program coordinators, directors, staff, and members of the Positive Living Society of British Columbia. We would also like to thank the Canadian Foundation for Dental Hygiene Research and Education (CFDHRE) for the financial support provided to this project.

22. Long-Term Periodontal Changes in Treatment with Oral Appliances

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Objectives: This retrospective, *in vivo*, clinical, and cephalometric observational study evaluated the periodontal changes associated with proclination of the mandibular incisors in individuals with obstructive sleep apnea (OSA) following the use of an oral appliance (OAm) for 4.5 or more years.

Methods: This study was conducted at two locations – UBC and a private practice office. Between 2004 and 2014, 145 consecutively treated patients at the UBC sleep apnea dental clinic were screened for specified inclusion and exclusion criteria. Patients were also recruited from a private practice. Informed consent was obtained. **A** clinical periodontal exam was performed by one investigator in addition to lateral cephalogram and maxillary and mandibular impressions. The periodontal exam included PSR (periodontal screening and recording), plaque index (Silness and Loë), gingival bleeding index (Ainamo and Bay), probing pocket depths and facial gingival margin thickness for mandibular anterior teeth in addition to clinical attachment level (CAL), recession and width of attached gingiva. Data (lateral cephalogram and study models) from baseline records (T0) and a recent visit (T1) were compared. Descriptive statistics, paired *t* test and intra-examiner calibration were used to analyze the data.

Results: Currently, data collection has been completed for 14 patients. Multiple patients (128) were excluded primarily due to the fact they could not be contacted, had switched to other treatment alternatives, or were missing baseline data. Approximately 10 additional patients have been scheduled and will participate in follow-ups accordingly.

Conclusions: Based on preliminary data we observed: (i) Clinically significant proclination of mandibular incisors with the continued long-term use of OAm; (ii) Clinical crown height did not change over the evaluated time period of >7 years despite the significant proclination of mandibular incisors; (iii) Gingival levels were maintained with clinically insignificant changes taking place during the study period.

23. External Cervical Root Resorption: Determinants and Treatment Outcomes

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Objectives: External cervical root resorption (ECRR) is a poorly investigated, dynamic process. The objectives of this study were to assess if any systemic condition is a potential predisposing factor for ECRR, and to examine the long-term ECRR treatment outcomes and its determinants.

Methods: This study contains data from 76 patients (98 teeth) diagnosed with ECRR at the UBC Graduate Endodontics clinic, from 2008 to 2018. Data regarding the medical and dental history were retrospectively collected from the charts of the ECRR group and an equivalent group of patients without ECRR (control group). Subsequently, the ECRR patients were approached for a follow-up appointment and the treatment outcome was documented. Chi-square/Fisher's exact tests were used for analyses at two levels (patient-based and tooth-based). The Kaplan-Meier curves method evaluated the overall ECRR survival/failure rates, and how local and treatment-related determinants were associated with the ECRR treatment outcome.

Results: Overall, 67 patients (89 teeth) were evaluated (88.2% patient follow-up rate and 90.8% tooth follow-up rate). The mean follow-up was 3.9 years, with the minimum follow-up being 1 year. The majority of the patients were older than 40 years old (72.4%). The most frequently affected teeth were the maxillary anteriors (31.7%), and the most common diagnosis was Class 2 (38.8%). Half of the cases survived for 8 years. Twenty-four teeth failed (i.e. 19 extracted, 5 not functional). The significant predictors were: diabetes (among systemic conditions), root canal treatment (RCT) and ECRR repair combined with RCT (among local determinants), and tooth location and the Heithersay classification (among treatment-related determinants).

Conclusions: Diabetes is a potential systemic predisposing factor for ECRR. RCT and the ECRR repair combined with RCT are associated with lower failure rates. Higher failure rates are associated with posterior teeth and higher classes in the Heithersay classification.

24. Expression Patterns of NF-kappaB in Potentially Malignant Inflammatory Oral Lesions

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Objectives: The malignant potential of oral lichen planus (OLP) remains controversial. Research has demonstrated that activation of inflammatory mediator nuclear factor-κB (NF-κB) is key to cancer development. There is a need to investigate NF-κB in oral potentially malignant lesions (OPML). In this study, we aim to compare clinical and risk habit differences between OLP and lichenoid dysplasia (LD), and determine if NF-κB expression is associated with features of OPML indicative of cancer risk.

Methods: Clinical, demographic, risk habit, and histological data have been collected from the Oral Cancer Prediction Longitudinal (OCPL) study and the CoPath Vancouver Coastal Health Database. Patients with a primary diagnosis of OLP or low-grade LD, no previous history of head and neck cancer, and at least 1 year of follow-up were eligible to participate. For completed cases, immunohistochemistry has been performed on formalin-fixed, paraffin-embedded tissue. Nuclear reactivity of NF-κB in the epithelium was counted in 10 high-power fields, and cytoplasmic positivity classified into four categories. Chi-squared tests were performed on categorical demographic and risk-habit data.

Results: Thirty-seven patients with OLP and 14 with LD have been recruited into this ongoing study. There is no significant difference in gender and age between groups ($p=0.297$, $p=0.120$, respectively). Smoking and lesion location at a high-risk site were significantly associated with a diagnosis of LD compared to OLP ($p=0.002$, $p<0.001$, respectively). Preliminary results show strong to moderate cytoplasmic reactivity in the epithelium and an increase in nuclear reactivity from OLP to dysplasia.

Conclusions: Patients with LD were more apt to be smokers and present with lesions at high-risk sites compared to those with OLP. Strong NF-κB cytoplasmic positivity, especially adjacent to intense inflammatory infiltrate, reinforces the prominent role of NF-κB in inflammation.

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25. Craniofacial Form Differences Between Obese and Non-Obese Orthodontic Patients

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Objectives: Although childhood obesity has recently seen a plateau in the US and Canada, it still affects 10-20% of children and adolescents. Increasing evidence points to obesity contributing to changes in growth and development, puberty, bone metabolism, and tooth movement. For this study, we aim to compare the craniofacial differences of obese and non-obese orthodontic patients between the ages of 6-12 years old, focusing on a younger age cohort than has been studied previously.

Methods: Height and weight measurements, age, and lateral cephalometric radiographs were gathered from patients, before beginning orthodontic treatment at the University of British Columbia. A group of 24 obese orthodontic patients were age, sex and angle-classification of malocclusion matched, with normal weight controls. Cephalometric radiographs were annotated and coordinates of landmarks were used to obtain traditional linear and angular measurements. Additionally, landmark coordinates were also subject to Geometric Morphometric (GM) analyses, such as Principle Component analysis and Discriminate Function analysis (MorphoJ), to ascertain whether differences exist between the craniofacial form in each group.

Results: Morphometric analysis of cephalograms taken from obese and non-obese children reveal subtle but significant differences between groups when assessing select linear and angular measurements. Our data corroborates but does not perfectly match previous studies assessing craniofacial differences between similar cohorts in older populations. GM analyses reveal a statistically significant difference in overall craniofacial form, between the obese and non-obese populations.

Conclusions: Our data point to a correlation between craniofacial form and physiologic, as well as metabolic, phenotypes of individuals. It also supports the need to include Body Mass Index records as part of a normal orthodontic assessment, to aid in treatment planning decisions such as timing of treatment and use of growth modification appliances.

26. Retrospective Review of Biodentine Pulpotomy Outcomes in Primary Molars

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Objectives: This study aimed to determine the effectiveness of Biodentine, a calcium-silicate material, as a pulpal medicament for primary molars requiring a pulpotomy procedure.

Methods: A retrospective chart review was conducted on children who received a Biodentine pulpotomy procedure on one or more primary molar(s) while receiving dental rehabilitation under general anesthesia from January 1, 2013 to May 1, 2018. Five clinical and radiographic outcomes were used to determine the success of the pulpotomy. The teeth were evaluated at intermittent recalls for up to 30 months post-treatment. Survival curves of the Biodentine pulpotomized teeth were estimated by nonparametric maximum likelihood methods for interval censored data.

Results: A total of 608 teeth from 208 children were evaluated over a 30-month post-treatment period. There was a total of 25 teeth with a failed pulpotomy procedure over the study period. Seven teeth were identified as having both a clinical and radiographic failure. Ten of the 608 teeth demonstrated clinical failure. Twenty-two of the 267 teeth with diagnostic radiographs demonstrated radiographic failure. A survival analysis curve indicated that the overall cumulative probability of survival remained at 97.2% over 30 months post-treatment.

Conclusions: Pulpotomy procedures on primary molars utilizing Biodentine as the pulpal medicament had favourable clinical and radiographic results after 30 months.

27. Positive Secular Changes in Dental Maturation Over a 30-Year Period

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Objectives: Secular change refers to the changes in rate of growth and development over successive generations. Evidence of secular change has been described across a multitude of populations. We currently use the methods of Logan and Kronfeld (1933) to assess dental age in the clinic, our aim was to test whether new, up-to-date Canadian standards need to be developed.

Methods: A retrospective chart review was carried out for children aged 6 to 13. Panoramic radiographs were examined for children between ages 6 and 13 in two cohorts, a historical group born between 1969 and 1980 and a recent group born between 2004 and 2011 (220 in each group). Root development on seven mandibular teeth was scored using the Demirjian system (1973). To assess changes in the timing of dental maturation, the median age of attainment for each stage of maturation was calculated using logistic regression. Boys were significantly less advanced compared to girls, thus comparisons of the historical and recent data were carried out on each sex separately.

Results: The statistical analysis by tooth type and stage revealed that the modern sample was significantly younger than the historical sample for a given tooth type and stage. Modern girls were, on average, 1 year ahead for the first molar root completion and up to 2 years ahead for completion of the first premolar root. Modern boys were also almost 1 year ahead for first molar root completion and 17 months ahead for the first premolar root completion. The recent boys and girls matured on average 0.58 years and 0.76 years earlier than their historical counterparts, respectively ($P<0.001$).

Conclusions: Positive secular changes have taken place in British Columbia during the last 30 years and tooth maturation is a sensitive way in which to measure childhood development.

Acknowledgements: Funded by the UBC Faculty of Dentistry. Human Ethics Approval - H1702181.

28. The Role of Granzyme B in Periodontal Connective Tissue Degradation

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Objectives: Periodontitis is an infectious disease resulting in inflammation within supporting tissues of teeth, with progressive attachment and bone loss. Tissue destruction during periodontitis is due to collagen-degrading matrix metalloproteinases (MMPs) released by resident cells of the periodontium in response to proinflammatory cytokines. Granzyme B (GzmB) is a serine protease produced by immune cells during chronic inflammation. GzmB is known to induce apoptosis cleavage, activate pro-inflammatory cytokines, and is involved in extracellular matrix protein destruction. However, the role of GzmB in the the pathogenesis of periodontitis is undefined. The aim of this study was to explore the role of GzmB in progression of periodontitis. We hypothesized that the level of GzmB is associated with chronic periodontitis by promoting the degradation of the connective tissues.

Methods: Human gingival fibroblasts (HGF) were treated with increasing concentrations of recombinant GzmB (from 0.5-10 nM) for time periods ranging from 0 to 24 h. The time course of MMP-1 release and activity in HGF supernatants were measured by enzyme-linked immunosorbent assay (ELISA). The cytotoxicity of GzmB was assessed using an MTS cell viability assay. In a study involving human subjects, gingival crevicular fluid (GCF) samples were obtained from sites of healthy periodontium, gingivitis, and periodontitis. GzmB was quantified in the GCF by ELISA.

Results: HGFs released more pro-MMP-1 in response to GzmB. Exposure to 10 nM GzmB caused a 3- to 4-fold increase in MMP-1 activity and secretion from cultured HGFs. The cell viability assay data showed that Granzyme B had no effect on HGFs viability. GzmB levels were higher in patients with severe periodontitis and gingivitis compared with healthy periodontium.

Conclusions: Gingival connective tissue degradation is associated with increased GzmB levels, suggesting a potential role for GzmB in the pathogenesis of periodontal disease.

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29. Fit of E-max and Celtra Duo CEREC Crowns

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Objectives: To compare marginal chipping, internal and marginal fit of E-max (EM) and Celtra Duo (CD) all-ceramic crowns fabricated using CEREC Omnicam and a chair-side milling chamber (CEREC MC XL).

Methods: Tooth #15 was prepared for all-ceramic crowns on an ivorine typodont. The maxillary typodont was then digitized using a CEREC Omnicam intraoral scanner and the STL file was used to mill a master model made from epoxy resin with 30 replica of tooth #15. A digital impression of the sectioned maxillary arch was recorded using CEREC. A total of 15 CD crowns and 15 EM crowns were designed chair-side and fabricated using a CEREC MC XL milling chamber. Micro-computed tomography (micro-CT) was utilized to assess the internal and marginal fit of crowns in both groups. Internal fit was assessed at eight selected points/crown. Marginal gap (MG) measurements were done at 20 systematically selected points/crown. The incidence of different marginal discrepancies were also recorded, including overextension (OE), underextension (UE), and chipping. Crowns with vertical MG >120 µm at more than five points were considered unacceptable and were rejected.

Results: CD crowns showed smaller cement space than EM crowns, 41 µm and 56.8 µm, respectively. CD crowns showed smaller mean vertical MG (47 µm) as compared to EM crowns (75 µm). The incidence of chipping was higher in the EM (53%) crowns than in the CD (27%) crowns. Overall, two crowns in the EM group were deemed unacceptable based on the MG measurements, while all crowns in the CD group were deemed acceptable.

Conclusions: The CD crown group resulted in better marginal fit and less chipping compared to the EM crowns fabricated using CEREC technology.

30. Ethylenediaminetetraacetic Acid Pre-Treatment Effect on Biofilm Dissolution by Sodium Hypochlorite

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Objectives: Endodontic treatment is aimed at the management and treatment of pathoses caused by bacterial colonization of the root canal system; this is primarily achieved through mechanical and chemical means. Various irrigants have been introduced in varying degrees, concentrations, and combinations to optimize bacterial eradication; however, there is no well-established optimal protocol, nor a recommended procedure by a regulatory body. The present study aims to evaluate the effect of ethylenediaminetetraacetic acid (EDTA) pre-treatment on sodium hypochlorite's ability to dissolve biofilm.

Methods: Hydroxyapatite discs were inoculated with subgingival plaque (Ethics Approval H12-02430) and biofilm was grown in brain-heart infusion broth for 3 weeks in anaerobic conditions. Samples were exposed to a pre-treatment of either sterile water or 17% ethylenediaminetetraacetic acid for 30 or 60 seconds, washed with an intermediate sterile water rinse, and subjected to either 2% or 6% sodium hypochlorite. Samples were examined under stereomicroscopy for complete dissolution of the biofilm; the time was recorded from initial exposure to sodium hypochlorite to complete dissolution.

Results: Biofilm dissolution time was decreased by increasing the concentration of sodium hypochlorite from 2% to 6% for all matched groups. Pre-treatment with 17% EDTA for 30 seconds decreased the time for biofilm dissolution by sodium hypochlorite while pre-treatment with 17% EDTA for 60 seconds increased the time for biofilm dissolution; this effect was conserved between both 2% and 6% concentrations of sodium hypochlorite.

Conclusions: Pre-treatment of biofilms with 17% EDTA will alter sodium hypochlorite's dissolution ability, as shorter exposure times may augment this dissolution while long exposure times may be detrimental to sodium hypochlorite's dissolution ability. Regardless of the length of pre-treatment time, sodium hypochlorite at both 2% and 6% concentrations was eventually able to completely dissolve all of the biofilm in all of the samples.

31. Effects of Irrigation and Agitation on Apical Vapor Lock

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Objectives: The objectives of the study were: 1) To detect the presence of apical vapor lock after positive pressure irrigation at two needle insertion depths. 2) To evaluate the elimination of apical vapor lock by manual dynamic agitation. 3) To investigate the replacement of the contrast solution with sodium hypochlorite at two needle insertion depths and flow rates.

Methodology: Twenty-eight single rooted teeth were shaped with either Vortex Blue 25/04 or ProTaper Gold F2 (25/08) rotary files. The presence of apical vapor lock was detected radiographically using a contrast mixture (sodium hypochlorite & cesium chloride) as the irrigant. Manual dynamic agitation with 50 strokes of a well-fitting gutta percha cone was performed in teeth with apical vapor lock. Its elimination was then evaluated radiographically. In teeth in which apical vapor lock was eliminated, replacement of the contrast solution with sodium hypochlorite was assessed radiographically. Each tooth was then shaped with Vortex Blue 30/04 or ProTaper Gold F3 (30/09) rotary files and the aforementioned experiments were repeated.

Results: Apical vapor lock was detected in 92.0% of the samples. Manual dynamic agitation eliminated the apical vapor lock in 81.6% of the teeth. Apical vapor lock was more likely to be present and eliminated in teeth that were shaped with ProTaper Gold rotary files. Increase in the flow rate, increase in the needle insertion depth, shaping with Vortex Blue rotary files, and smaller apical preparation size improved the replacement of the contrast solution.

Conclusions: Within the limitations of this study, shaping with ProTaper Gold rotary files has a joint effect in the formation and elimination of apical vapor lock. Replacement of the contrast solution with sodium hypochlorite was affected by the independent effect of needle insertion depth and flow rate and the joint effect of needle insertion depth, flow rate, rotary file system, and apical size preparation.

32. Effect of Crown Cementation on Marginal Fit Using Micro-CT Measurement

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Objectives: To investigate the effect of intaglio adjustment and cementation on the marginal fit of lithium disilicate (LDS) crowns fabricated using traditional impression and manufacturing (TP), digital impression and traditional pressed manufacturing (DP), and digital impression and manufacturing (DD).

Methods: An Ivorine typodont (tooth #15) was prepared for an all-ceramic crown. The prepared tooth was scanned, and 45 dies produced for use in the fabrication of LDS crowns using three techniques: DD, DP, and TP. Micro-computed tomography (micro-CT) was utilized to assess the 2D marginal fit of crowns in all three groups before adjustment, after adjustment, and after cementation. RelyX-Ultimate resin cement was used to cement the crowns on each of the 45 dies with 20 N application force using the universal testing machine. The 2D vertical marginal gap (MG) measurements were done at 20 systematically selected positions along the crown margins. The results were analyzed using a linear mixed effect model with the Method × Cementation interaction as the fixed effect and Crown as a random effect along with a post hoc test (CI=95%).

Results: A decrease in the marginal gap measurement was recorded as a result of clinical adjustments to crowns that were initially deemed to be clinically unacceptable. Cementation resulted in an average increase of 20 µm (95% CI= 18-22) in the marginal gap, irrespective of the method of fabrication.

Conclusions: Clinical adjustments to all crowns reduced marginal gap, while cementation significantly increased the marginal gap regardless of the method of fabrication.

33. Imaging of Murine Melanoma Tumours Using Fluorescent Gold Nanoparticles

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Objectives: Contrast agents are required to view and differentiate soft tissue structures in computed tomography (CT) yet in pre-clinical research, histology is still considered to be the gold standard. Pre-clinical contrast agents used for radiographic imaging are not visible when viewed histologically, nor are histological stains visible radiographically. By identifying a single agent that is visible in both x-ray and optical imaging, we can ensure that the target tissues can easily be identified and correlated in both images, without the need of additional staining techniques. Here we present an approach to allow for the correlation of imaging murine melanoma tumours using micro-computed tomography (micro-CT) and optical projection tomography (OPT), using fluorescently-labelled gold nanoparticles without additional tissue staining.

Methods: B16F10 cells, a murine melanoma cell line, were used to induce tumour growth in the right hind legs of twelve female C57Bl6 mice. Tumour growth times varied between 2-3 weeks, with a maximal tumour size of 1 cm^3. We injected Cy3 fluorescently-coated gold nanorods directly into the tumours prior to imaging. The mice were scanned with *in vivo* micro-CT (for pre- and post-contrast scans at a resolution of 50 microns). Once euthanized, the hind leg was dissected and scanned with a specimen micro-CT at a higher resolution of 10-17.5 microns. The dissected hind legs were fixed, embedded into agarose, cleared, and visualized under OPT using filtered UV light at 545-610 nm.

Results: Distribution of the gold nanoparticles appeared to be contained and isolated to the murine melanoma tumour, allowing for contrast and visualization under micro-CT. OPT imaging was less conclusive, as muscle tissue provides its own auto-fluorescence; therefore, correlation of the images proved difficult.

Conclusions: Fluorescently-labelled gold nanorods injected into murine melanoma tumours can be visualized under micro-CT imaging; however, challenges remain in being able to isolate the fluorescence of these same particles under OPT imaging.

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34. Reviving Image Cytometry System for Oral Cancer Screening

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Objectives: Cytology**,** like a cervical Pap smear, provides an excellent non-invasive tool for cancer screening. DNA image cytometry (DNA-ICM) systems were designed to detect gross alterations of nuclear DNA content representing chromosomal aneuploidy, a biomarker of malignancy. Previously published results using Feulgen-thionin-stained oral brushing samples, a Health Canada-approved imaging system, ClearCyte (Perceptronix Medical Inc.), and an experienced cytopathologist to assess the results, have demonstrated 89% sensitivity and 97% specificity. The purpose of this study was to develop a robust repeatable approach using the ClearCyte system for province-wide oral cancer screening.

Methods: A set of 114 abnormal oral brushing samples of high-grade dysplasia (n=34) and cancer (n=80) were retrieved from the biobank of a Pan-Canadian Surgical Trial. Normal oral brushings (n=20) were collected from the Oral Mucosal Clinic. The cells from these liquid brush biopsies were cytospun onto glass slides using a Cytospin 4 (Thermo Scientific, Waltham, MA), stained with Feulgen-thionin, and scanned and analyzed on a ClearCyte system. Specificity and sensitivity of the ClearCyte results were calculated.

Results: A total of 130 cases met inclusion criteria of ≥400 nuclei per case. Four (3.0%) oral cancer samples were excluded from analysis due to extremely low nuclei counts. Samples with detected aneuploid cells, ≥10% tetraploid cells, or ≥5% hyperdiploid cells were considered abnormal. The sensitivity, specificity, negative predictive, and positive predictive values were 69.1%, 90.0%, 34.6%, and 97.4%, respectively.

Conclusions: Although DNA-ICM provides a potentially robust, non-invasive oral cancer screening technique, the manual input necessary for quality assurance of the ClearCyte test proved to be excessive. Further refinement and automation of the system by introducing new algorithms will help reduce the workload of cytotechnicians, minimize the need for cytopathologist review, breach the geographical barrier between remote communities and oral pathologists, reduce the number of unnecessary biopsies, and save lives through earlier cancer detection.

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35. Pregnant Women's Views on Integrating Oral Health into Prenatal Care

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Objectives: Women's oral health has been associated with fetal health, pregnancy outcomes and the future child's oral health status; preventive oral healthcare is therefore recommended during prenatal care, particularly among vulnerable pregnant women e.g. the working poor. However, there is little information on pregnant women's preferences concerning oral health care service delivery during prenatal care. This study therefore sought to explore the views of vulnerable women on the establishment of community-based preventive dental services in a maternity facility.

Methods: Fifteen women participated in three audio-recorded focus group discussions in a maternity clinic for vulnerable women in Surrey, BC. Discussions were transcribed verbatim and a thematic analysis to identify main themes about community-based preventive services was conducted. The four main themes were discussed by the authors until consensus.

Results: All pregnant women reported 1) unmet oral health care needs and 2) difficulty with accessing this care because of high cost and lack of insurance. In terms of 3) prenatal oral health information, participants favoured the provision of oral healthcare as part of routine prenatal care. Their primary interest was in receiving accurate preventive oral health information, either from dental professionals or other prenatal health care team members. The 4) most-needed care services, identified by participants, were dental screening, diagnosis and oral hygiene therapy, and treatment at a reduced cost. A few suggested the extension of these services until one year after delivery.

Conclusions: Vulnerable pregnant women have unmet dental needs and are positively disposed to the provision of community-based primary preventive dental services as part of prenatal care, either from dental professionals or other prenatal health care team members.

36. Fit of Zirconia Crowns Using Different Preparation Designs and Sintering

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Objectives: Marginal fit is an essential component for the clinical success of dental restorations. The aim of this study was to investigate the influence of different finish line widths and crown thicknesses on the marginal fit of zirconia crowns fabricated using either standard or fast sintering protocols.

Methods: Six titanium abutments were fabricated for receiving zirconia molar crowns. Crowns were designed virtually and milled from partially sintered zirconia blanks *(IPS e.max ZirCAD)* and divided into 12 groups (n=10/group). Crowns in groups 1 to 6 were sintered by standard sintering, while those in groups 7 to 12 were sintered by fast sintering: G1/G7 (0.5mm chamfer, 0.8mm thickness); G2/G8 (0.5mm chamfer, 1.5mm thickness); G3/G9 (1.0mm chamfer, 0.8mm thickness); G4/10 (1.0mm chamfer, 1.5mm thickness); G5/G11 (1.2mm chamfer, 0.8mm thickness); G6/G12 (1.2mm chamfer, 1.5mm thickness). The vertical marginal gaps were assessed at eight locations using digital microscopy.

Results: All vertical marginal gaps were within the clinically acceptable range (~11-52µm). G8 (FS, 0.5mm chamfer, 1.5mm thickness) demonstrated the largest gaps (47.95µm with 95% CI: 44.57, 51.23), whereas G3 (SS, 1.0mm chamfer, 0.8mm thickness) had the smallest marginal gap (14.43µm with 95% CI: 11.15, 17.71). A linear mixed effect model showed significant differences for the interaction between finish line × crown thickness × sintering ($p<0.001$). The lingual surfaces showed the largest gaps in both sintering protocols, while the mesial and mesiobuccal surfaces demonstrated the smallest gaps.

Conclusions: There was significant interaction between finish line widths, crown thickness, and sintering protocol on the vertical marginal gaps. A 1.0mm finish line with either 0.8mm or 1.5mm crown thickness had better marginal fit in both sintering protocols compared to 0.5mm or 1.2mm finish lines. Smaller marginal discrepancies were observed for standard compared to fast sintering except with a 0.5mm finish line and 0.8mm crown thickness.

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37. Validation of Hounsfield Unit Measurements in Dental Cone Beam CT

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Objectives: Hounsfield units (HU), in computed tomography (CT) x-ray images, have helped in the assessment of the overall density of materials and therefore detection of any abnormal changes. Hospital CT scanners use HU as standard of care; however, this tool is not available in any of the dental cone beam (CB) CT scanners. Many studies argued against the application of HU in dental CBCT due to some geometric factors. This study aims to explore a potential approach to introducing HU into dental CBCT.

Methods: Image analysis and measurement of the mean gray values for water and air phantoms and an image quality phantom (SEDENTEXCT IQ) were performed over a six-month period to check for the consistency and reliability of the dental CBCT (Carestream 9300). Then images were scaled according to a manual script using MATLAB software and measured values for air and water, in order to rescale the image into HU. HU scaled images were compared to images obtained from hospital-based medical CT (Toshiba Aquilion ONE) for the same phantoms.

Results: The mean gray values of the dental CBCT images after the application of the HU scripts were shifted more toward the expected HU values for most of the materials compared to the original images. HU values obtained from medical CBCT were closer to the expected HU values in comparison to images acquired from the dental CBCT.

Conclusions: HU number is a crucial tool for the diagnosis of many abnormalities. The result of this study showed a promising method to obtain a reliable HU number after application of some scripts to CBCT images.

38. Conducting Community Oral Cancer Screening Among South Asians in BC

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Objectives: Globally, more than 300,000 cases of oral cancer are diagnosed annually. South Asian countries, like India, have a high incidence due to rampant use of chewing tobacco. The majority of oral cancers develop from oral potentially malignant lesions (OPML). Oral cancer screenings can reduce mortality rates substantially. It can be challenging for clinicians to differentiate benign lesions from OPML. Quantitative cytology (QC), which studies DNA ploidy and nuclear morphometric features has shown to be an effective predictor of malignant transformation in OPML. The aim of this project is to assess the need for oral cancer screening among South Asians in BC and to validate QC as an adjunct screening device to aid in identifying high-risk lesions among visually suspicious lesions.

Methods: Demographic information (gender, age, ethnicity, risk habits and dental usage) were collected. Extraoral, intraoral, and fluorescence visualization (FV) examinations were conducted. Buccal mucosal samples along with brushings from lesions or areas of loss of fluorescence were collected. Thin-layer cytology slides were prepared and stained using Feulgen Eosin. Slides were scanned using the Cancer Imaging Scanner at BC Cancer which uses machine learning classification algorithms to classify cells based on QC and malignancy associated changes.

Results: Out of 307 participants screened, 303 were eligible. 104 (34%) lesions were documented: 45 (15%) high risk (i.e. white/red lesions, lichen planus) and 59 (19%) low risk (i.e. candidiasis, aphthous ulcers). Of these, 12 were referred to the Next Gen clinic for biopsy, while 8 were reassessed at 3 weeks. Chewing tobacco was found to be associated with lesion presence ($p<0.001$). QC is ongoing for 320 samples. To date, 5 biopsies have been performed resulting in 1 mild and 1 severe dysplasia.

Conclusions: South Asians in BC were found to be at high risk for OPMLs. QC may help to improve the sensitivity and specificity of oral cancer screenings.

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39. Maternal Psychosocial Factors and Dental Utilization for Special Needs Youth

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Objectives: Parents of youth with special healthcare needs (YSHCN) face barriers to dental service utilization due to psychosocial factors. Additionally, caring for YSHCN increases parental stress which may further impact their utilization. The aim was to evaluate the impact of maternal psychosocial factors on dental service utilization in youth with special health care needs.

Methods: We analyzed data among youth aged 12-17 years using the 2011/2012 US National Survey of Children's Health (n= 31,840). Descriptive analyses compared YSHCN with non-YSHCN. Multivariable logistic regression models tested associations between maternal psychosocial factors (family income, maternal education, and parenting stress) and two dental care outcomes: preventive dental care use and unmet dental care.

Results: Overall, similar proportions of youth (~ 85.0%) in both groups used preventive dental care in the previous 12 months. A higher proportion of YSHCN reported unmet dental needs compared to non-YSHCN; 5.9% vs. 3.4%, respectively. Compared to parents of non-YSHCN (8.9 %), parents of YSHCN reported higher parenting stress (22.2%). In both groups, family income, not maternal education, were positively associated with dental service utilization. In the YSHCN group, higher parenting stress was associated with higher levels of unmet dental care (Adjusted Odds Ratio [AOR]=1.8; $p<0.05$) and lower levels of preventive dental care use (AOR=0.7; $p<0.05$).

Conclusions: The majority of parents reported that their children received preventive dental care and had good oral health, yet have unmet dental needs; however, income-related disparities in dental utilization still exist. Parenting stress was a determinant of dental utilization for the YSHCN and helped in explaining the relationship between other family-level determinants and dental utilization.

40. Evaluating a Community Dental Hygiene Clinic for Justice-Involved Women

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Objectives: Women involved with the criminal justice system face oral health inequalities and inequities in access to oral care. In collaboration with the Elizabeth Fry Society of Greater Vancouver (EFry), an organization that supports justice-involved women, dental hygiene students from the University of British Columbia provide on-site preventive oral care for EFry clients. To inform the design of a prospective full-service dental clinic for EFry clients, this study aimed to evaluate 1) how the clinic has met the oral health needs of clients, and 2) how the clinic has facilitated access to oral care.

Methods: A multi-method approach was used. Clients' dental charts were reviewed retrospectively to describe client demographics, oral health status, services provided, and clinic utilization, and univariate, descriptive statistical analyses were performed. A review of client satisfaction surveys and clinic observation was conducted to inform interview questions. Three focus groups and three interviews with twelve clients and one focus group with four EFry staff was conducted to explore aspects of the clinic that facilitated access, they were audio-recorded, transcribed verbatim, and analyzed thematically.

Results: Ninety-three women and six children received preventive oral care over 3 years. Services provided addressed clients' preventive oral health needs and the majority of clients received an oral assessment, periodontal therapy, fluoride varnish, and oral health education. The affordability and convenience of the clinic and the respectful, attentive, and non-judgmental care facilitated access. Accommodation factors including timely and effective communication between clients and students and clinic promotional material that de-emphasizes the university's involvement should be considered in the design of the prospective clinic.

Conclusions: This outreach program facilitated access to oral care for justice-involved women, and EFry clients and staff value this program. The results of this study will inform the future clinic about how to appropriately provide oral care to this group.

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41. Lay Public and Dental Professional Knowledge Around HPV Transmission

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Objectives: Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide, but most infected individuals are unaware of the infection. HPV has also been associated with certain types of oral cancer, and yet, the level of knowledge that dental professionals have in terms of HPV transmission, oral sexual activities, and oral cancer development needs exploration. The aim of this study was to assess the knowledge held by dental professionals, as well as queer and straight lay public, regarding Human Papillomavirus (HPV) transmission through oral sex and oral cancer development.

Methods: Textual data were collected from a public forum with dental professionals in Vancouver who discussed the HPV-oral sex-oral cancer triad and from survey data gathered from 212 lay public participants (also in Vancouver) who answered a 13-item questionnaire on the perceived risks of oral sex in terms of HPV infection and oral cancer development. Data were analysed statistically by age group, gender, and sexual orientation, with descriptive statistics, while an ANOVA test was used to compare variation in the responses to the survey (*p*-value=0.05).

Results: The forum engaged 46 health care professionals who discussed the potential risks for head and neck cancer development through HPV infection, beyond tobacco smoking and excessive alcohol consumption. The survey revealed that 34.5% of the participants believed that oral sex is an activity of *no or low risk* for the transmission of HPV, while 84% of participants believed the same sexual practices were of *no or low risk* for HIV (Human Immunodeficiency Virus) transmission. Most participants (82%) never discussed oral sexual activities with their physicians or dentists/dental hygienists.

Conclusions: The potential links between HPV infection and oral cancer remain mostly unknown by the public. Physicians and dental providers should discuss oral sexual practices to raise awareness with their patients.

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42. Evolutionary History of the Periodontal Ligament: Implications for Dental Ankylosis

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Objectives: Mammals are unique in having a gomphosis, which is a tooth set in a socket and attached to the jaw with a periodontal ligament (PDL). However, mammalian ancestors lacked the PDL, and teeth were ankylosed to the jawbone. This study aims to examine the 300 million year evolutionary history of mammals preserved in the fossil record to determine when and how the PDL originated.

Methods: Thin sections of fossil mammalian ancestors (n=33) and living mammals (n=2) were made and examined with plain and cross-polarized light. Teeth were scored based on their developmental stage: 1) Erupting, 2) Gomphosis, 3) Mineralization, and 4) Ankylosis. The frequencies of these categories were mapped onto a phylogenetic tree to determine the evolutionary pattern of PDL development.

Results: Although the PDL does not fossilize, Sharpey's fibres present in the tooth cementum and alveolar bone indicate the PDL was present for varying degrees of time throughout the life of each tooth, even in animals with fully ankylosed teeth. Therefore, teeth pass through each developmental stage sequentially: eruption, gomphosis, mineralization, and ankylosis, and the PDL is maintained in mammals by delaying the processes that produced dental ankylosis in stem mammals.

Conclusions: Differences in tooth attachment in the evolution of mammals is created through differences in timing of tooth tissue development and the degree of PDL mineralization. Dental ankylosis was part of the normal development of the stem mammal periodontium for millions of years prior to the evolution of a permanent gomphosis in mammals. Pathological ankylosis may represent a reversion to the ancestral condition, which now only forms via advanced ageing and pathology.

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43. Effects of Bioactive Materials on Physical Properties of Dental Resin

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Objectives: Adhesive dentistry has improved minimally invasive restorative techniques. However, resinous materials can still be enhanced by the addition of antibacterial compounds, thus preventing secondary caries. This study aimed to verify the effects of two antibacterial compounds (undisclosed) on various physical properties of dental resin blends.

Methods: Experimental resin blends (50% BisEMA; 30% TeEGDMA; 14% HEMA; 4% ethanol; 2% photoinitiators) were loaded with one of two bioactive materials (BAM1 or BAM2), silanated (-S) or non-silanated (-NS) at 0.5%, 1.0%, 1.5%, or 3.0% by weight (n=10). Mixtures were stirred for 24h followed by 24h vacuum evaporation. The control group incorporated the resin blend only. Experimental materials were tested for flexural strength (FS), water sorption (WS), and solubility (S). FS specimens followed ISO 4049 and were stored in artificial saliva (24h, 37°C). For WS and S tests, specimens (4mm x 2mm) were analyzed after 28-day water incubation at 37ºC.

Results: For FS, BAM1-S (all concentrations) were statistically similar to the control ($p>0.05$). However, BAM1-NS at 0.5% and 1% showed lower FS compared to the control ($p<0.05$). For BAM2-S and -NS FS results, both presented lower values than the control, regardless of concentration ($p<0.05$). For WS results, BAM1-S and -NS (3%) showed statistically higher values than the control. BAM2-S at 0.5% and BAM2-NS at 1%, 1.5%, and 3% all showed higher WS than the control ($p<0.05$). Solubility results for BAM1-NS were all similar to the control; however, for BAM1-S at 0.5% and 1.5%, S was higher than the control. BAM2-NS at 3% and BAM2-S at 1%, 1.5%, and 3% results indicated higher S than the control ($p<0.05$).

Conclusions: Use of bioactive compounds to enhance antibacterial properties of dental resins can be considered as an alternative, depending on concentration and treatment, without significant changes to physical properties. Further investigations will assess the antibacterial properties of the resin blends.

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44. Effects of Over-The-Counter Bleaching Agents on Properties of Dental Enamel

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Objectives: Vital dental bleaching has recently presented an exponential increase in use due to its conservative approach to treat discoloured teeth. However, multiple bleaching products can be directly purchased by the patient (over-the-counter (OTC) or e-commerce) without professional supervision. Thus, this study aims to evaluate the effects of four OTC bleaching products on the properties of dental enamel.

Methods: Twenty human pre-molar crowns (REB H17-02331) were randomly assigned to four groups (N=5): **G1**:Poladay 9.5%HP (SDI), **G2**:White Teeth Global 44%CP (BleachPro Whitening), **G3**:Crest 3D White (Procter&Gamble); unknown HP%, and **G4**:Teeth Whitening Gel (HiSmile); unknown HP%. Each crown was sectioned into four quarters; segments were imbedded together into an epoxy resin block, flattened, and polished. Each quarter was treated with four different bleaching times as follows: control/no-bleaching, 2-week, 4-week, or 8-week. Bleaching materials were applied to enamel surfaces as per manufacturer's recommendations. Enamel surfaces were analyzed for ultra-micro hardness, elastic modulus, and superficial roughness. Each material was analyzed for hydrogen peroxide content by titration.

Results: Enamel surfaces treated with OTC bleaching agents presented ultra-micro hardness values significantly lower than the control ($p<0.05$) for all materials and treatment times, except G4 (HiSmile). The modulus of elasticity was significantly reduced after bleaching with G1 (Poladay) for 2 and 4 weeks, and with G4 (HiSmile) at 2 weeks ($p<0.05$). Surface roughness was significantly increased by bleaching time on G3 (Crest3DWhite) at 2 weeks ($p<0.05$). Hydrogen peroxide present on the materials tested were G1:11.46%, G2:14.1%, G3:3.31%, and G4:0.03%. Among materials, significantly lower ultra-micro hardness and higher surface roughness were observed for bleaching products with higher HP% ($p<0.05$), i.e., G1 (Poladay) and G2 (White Teeth Global).

Conclusions: Over-the-counter bleaching products containing hydrogen peroxide can significantly alter human dental enamel ultra-microhardness, modulus of elasticity, and surface roughness. The application time also affects those properties; however, it varies for each bleaching product.

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