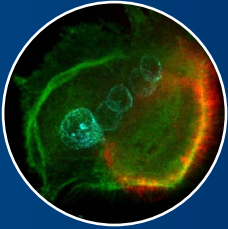




THE UNIVERSITY OF BRITISH COLUMBIA  
Faculty of Dentistry



# Research Day 2023

Clusters of Diverse Research Excellence

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# ABSTRACTS

## Water Sorption and Solubility of Resin Synergistically Loaded with Photosensitizers

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**Objectives:** Dental caries is one of the most prevalent chronic diseases and remains a significant global health and economic concern. Development of dental resins with antimicrobial photodynamic properties is a promising therapeutic approach to prevent recurrence of the disease and prolong the lifespan of restorations. The objective of this study was to evaluate the effect of adding two different photosensitizers, zinc oxide nanoparticles (ZnO-NPs) and riboflavin (B2), on water sorption (WS) and solubility (SL) of methacrylate-based resin blends.

**Methods:** Nine different dental resin formulations containing BisEMA (50%), TEEGDMA (30%), HEMA (14%), Ethanol (4%), and photoinitiators (2%), were loaded with ZnO-NPs (0, 5 and 10 wt%) and B2 (0, 1, and 1.5 wt%). Resin disks were light cured for 120 s at 1100 mW/cm<sup>2</sup> (n=5). A 10-day WS/SL analysis at 37°C in MQH<sub>2</sub>O, followed by a 7-day drying cycle, was carried out. Data were subjected to two-factor general linear model and post-hoc Tukey tests ( $\alpha=0.05$ ).

**Results:** While the presence of B2 in the resin blend had an overall significant impact on the WS of the blends ( $p<0.001$ ), only the 10% ZnO/1% B2 blend had a significant drop in WS compared to the 0/0 control blend ( $p<0.001$ ). Conversely, the inclusion of ZnO-NPs was found to have a significant impact on resin SL ( $p<0.001$ ), with resins containing 5wt% ZnO presenting higher SL compared to the 0/0 control blend ( $p<0.001$ ).

**Conclusions:** This study demonstrated the WS and SL impact of adding two different photosensitizers into a methacrylate-based resin blend. Adding 1.5 wt% B2 resulted in a decrease in SL for both the 0/0 control and 5wt% ZnO specimens. Future studies will consider optimizing the sample preparation protocol, such that less light energy is necessary for through-thickness cure.

**Acknowledgements:** Supported by the New Frontier in Research Fund, Government of Canada (NFRFE-2019-00061) and Dental Biomaterials Undergraduate Summer Research Award.

## Mapping the Teaching Cases at the Faculty of Dentistry

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**Objectives:** Dental schools should support diversity, equity, and inclusion within their teaching curricula. This study mapped out the Problem-Based Learning (PBL) and Dental Applied Learning Experience (DALE) cases students discussed at the UBC Faculty of Dentistry in terms of patients' characteristics and treatment outcomes.

**Methods:** PBL and DALE cases from the Fundamentals of Medical Sciences I and II, the two largest case-based modules, were mapped out based on patient characteristics (e.g., age, gender, ethnicity, sexual orientation, type of insurance), and treatment outcome (e.g., successful/unsuccessful). These characteristics were contrasted with the composition of the population in British Columbia (BC) and with the evidence on treatment outcomes.

**Results:** Thirty-four cases were mapped out of 58: 0.4% of them portrayed non-straight white patients (4% in BC); 9% portrayed patients older than 65 yrs (18% in BC); 26% of the cases included patients with some form of disability, but mostly in terms of heavy weight (37% in BC); 0% of them involved First Nations patients (6% in BC); 75% of the treatments were successful (successful rate can be as low as 50%); and 0% of the cases mentioned availability of dental insurance (30% of BC residents do not have dental insurance). Treatments involving patients without disability were 2.04 times more likely to be successful than those involving patient with a disability.

**Conclusions:** Characteristics of patients portrayed in the existing PBL and DALE cases seem to differ from the composition of the population in British Columbia. The cases students discuss should be revised to better portray the mosaic of British Columbians and treatment outcomes. Students should have more opportunities to actively think about the barriers to care that other groups and populations face based on their inherent characteristics, and to consider their own internalized biases and learn to refrain from stereotyping certain populations in practice.

## Effects of Photosensitizer Concentration and Monomer Type on Resin Photopolymerization

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**Objectives:** Dental caries is a multifactorial chronic disease resulting in debilitating complications and financial strain, and commonly recurs with current treatment modalities. To address the lack of long-term and effective strategies for treating dental caries, we pursued the development of methacrylate-based dental resin blends simultaneously loaded with two different photosensitive compounds. This study evaluated the effect of loading ZnO nanoparticles (ZnO-NPs) and riboflavin (B2) on the degree of conversion (DC) for two experimental dental resin blends (RB): one based on BisEMA and another on BisGMA. The goal is to create a material with a carefully tuned composition for use in an antimicrobial photodynamic therapy-based approach to prevent recurrent dental caries.

**Methods:** From two experimental RBs, BisEMA- and BisGMA-based, 12 formulations for each experimental blend were produced using 50–150nm ZnO-NPs (0, 5, 10, 20wt%) and B2 (0, 1.0, 1.5wt%). Resins were analyzed by FTIR for DC after 30s and 60s curing time (1300mW/cm<sup>2</sup>; n=6). Data were subjected to a univariate general linear model and post-hoc Tukey ( $\alpha=0.05$ ) tests.

**Results:** Concentrations of ZnO-NPs and B2 were both found to detectably impact DC following either 30s or 60s of light curing ( $p<0.001$ ). In general, as B2 concentration increased and the ZnO-NP concentration decreased, DC increased regardless of curing time. Meanwhile, although the choice of main monomer in the blend (i.e. BisGMA or BisEMA) had a significant overall impact on DC ( $p<0.001$ ), there were few pairwise comparisons that were significant for this independent variable.

**Conclusions:** This study demonstrated the potential to tune the DC of experimental RBs by selectively adding ZnO-NP and/or B2 to the formulation. Overall, adding B2 to the blend can help reverse any drop in DC caused by adding up to 10wt% ZnO-NPs. Future studies will investigate how this dual loading of different photosensitizers can have a synergistic antimicrobial response on select microorganisms.

**Acknowledgements:** Supported by the New Frontiers in Research Fund, Government of Canada (NFRFE-2019-00061) and UBC Faculty of Dentistry Undergraduate Student Summer Research Award.

## Prediction of Clinical Aggressiveness of Oral Cancer Using Quantitative Pathology

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**Objectives:** Oral squamous cell carcinoma (OSCC) is a deadly disease with a low survival rate of 60-65%, in which early-stage patients have a one-in-four risk of regional metastasis (LN+) and no validated biomarker(s) to predict LN+ in early-stage OSCC. In this study, we explored 156 quantitative nuclear phenotypes (QNP)—constituting nuclear morphology, DNA amount, and chromatin organization—on OSCC biopsy (BX) and surgical (ST) tissues to identify which features will be helpful in determining the probability of LN+. The research question is whether the BX and ST samples attribute to similar QNP and whether QNP can distinguish LN+ and LN0 OSCCs.

**Methods:** A retrospective study was conducted on patients diagnosed with OSCC from a pan-Canadian surgical trial. As a proof of principle, BX and ST from two patients (LN+ and LN0) were sectioned, stained, and scanned: one slide with hematoxylin–and-eosin (HE) staining for cellular morphology and another slide with Feulgen-thionin (FT) staining for QNP analysis. An in-house imaging histology software was used to analyze tumoural regions of interest and to analyze 156 features of the extracted cells.

**Results:** A total of 32,887 nuclei were analyzed. Using Wilcoxon tests, over 100 features were significantly different ( $p < 0.05$ ) between BX and ST of the same patient or of the two patients. When comparing features between LN0 and LN+, there were significant differences among either the BX or ST. These features were around Morphology, Photometric, Discrete, Markovian, and Fractal of QNP.

**Conclusions:** The QNP analysis showed differences between BX and ST and potential ability to discriminate LN+ from LN0 cases, with the former highlighting the potential variability of obtaining biopsies. More samples are required to build prediction models and to determine which of the BX or ST performs better in prediction of LN in early-stage OSCC.

**Acknowledgements:** Supported by BC Cancer Foundation and UBC Faculty of Dentistry Undergraduate Student Summer Research Award.

## **Monoamine Oxidase Inhibitors and Their Regulatory Effect on LPS-Induced Osteoclastogenesis**

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**Objectives:** Lipopolysaccharide (LPS) is a component of the outer membrane of all gram-negative bacteria and proposed to be a potent inducer of bone resorption in inflammatory bone diseases, such as periodontitis, via the production of inflammatory cytokines. Previous findings have implicated that the monoamine oxidase (MAO) A/B genes are upregulated in chronic inflammatory states including periodontitis. Thus, the Putnins lab developed a novel MAO inhibitor—RG0216—with reduced blood-brain barrier permeability and anti-inflammatory effects. We hypothesize that RG0216 inhibition of LPS-induced osteoclastogenesis is mediated by downregulation of the inflammatory cytokine, TNF- $\alpha$ . Using cell culture approaches we examined RG0216 regulatory effects on LPS-induced osteoclastogenesis and its effects on TNF- $\alpha$  expression.

**Methods:** The murine RAW 264.7 cell line was treated with recombinant RANKL protein to induce osteoclastogenesis. To assess the effect of LPS and RG0216 on osteoclastogenesis *in vitro*, cultures were treated with *E. coli* O55:B5 LPS using different concentrations and time parameters. RNA was extracted, cDNA prepared, and relative gene expression of tartrate-resistant acid phosphatase (TRAP), cathepsin K, and TNF- $\alpha$  were examined using qRT-PCR. TRAP staining of cultures and cell proliferation assays were performed in parallel. Changes in LPS induction of osteoclastogenesis was examined in the presence of RG0216 and TNF- $\alpha$  blocking antibody. TNF- $\alpha$  protein in conditioned media was assayed using ELISA (Mesoscale).

**Results:** LPS induced osteoclastogenesis in cells pretreated with RANKL. RG0216 and TNF- $\alpha$  blocking antibody reduced the number of TRAP<sup>+</sup> cells in LPS/RANKL-treated cultures and RG0216 reduced TNF- $\alpha$  gene and protein expression.

**Conclusions:** LPS induced osteoclastogenesis in RANKL pretreated cells. The MAO inhibitor RG0216 decreased TNF- $\alpha$  expression and this was associated with a decrease in the number of osteoclasts seen in the cell culture studies. RG0216 shows promise as a potential future therapeutic treatment for periodontitis; however, further research is needed to understand the regulatory mechanisms mediating these effects.

**Acknowledgements:** We recognize the UBC Faculty of Dentistry Undergraduate Student Summer Research Award and CIHR for project support.

## **DNA ploidy change over time and malignant transformation**

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**Objectives:** Most oral cancers derive from oral potentially malignant lesions (OPML). The gold standard to predict malignant transformation (MT) is grade of dysplasia. It is difficult to assess risk of MT of low-grade OPML. DNA image cytometry (DNA-ICM) uses cells collected by brushings, a non-invasive, painless, and less expensive way to assess risk of MT. This study aimed to analyze the clinical characteristics of lesions associated with repeated lesion brushing analyzed for ploidy in patients with biopsy-proven low-grade dysplasia, in addition to determining if lesions can be triaged by risk of progression based on ploidy and clinical features.

**Methods:** Data and samples used in this nested case series were from subjects enrolled in the Oral Cancer Prediction Longitudinal Study. Eligibility criteria includes a histologically confirmed diagnosis of mild or moderate dysplasia, a minimum of 5 years of follow-up for non-progressing cases, and a minimum of a lesion brushing at two different time points in follow-up. Progressors (P) are biopsy confirmed low-grade dysplasia that progressed to high-grade dysplasia or squamous cell carcinoma (SCC); and non-progressors (NP) are low-grade dysplasias that did not progress to high-grade dysplasia or SCC.

**Results:** Thirty-eight cases, 12 P and 26 NP, of low-grade dysplasia with multiple DNA-ICM results were analyzed. Sixteen NP lesions had no aneuploid cells, while eight had a single instance of aneuploidy that resolved following biopsy. Two NP cases had aneuploid cells throughout follow-up: one patient died and the second was treated while still a moderate dysplasia. Of the 12 P cases in follow-up, five had no aneuploid cells and seven showed aneuploidy: all had high-risk clinical signs (size, appearance, or site).

**Conclusions:** DNA-ICM using cells from brushings collected over time in combination with clinical risk factors may serve as a tool to identify high-risk OPML and to note change in OPML risk.

**Acknowledgements:** Supported by Cancer Control Research with BC Cancer and a UBC Faculty of Dentistry Undergraduate Student Summer Research Award.

## The Role of Microtubules in Platelet Secretion

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**Objectives:** Platelets are small blood cells responsible for hemostasis and wound healing. Platelets are activated upon interaction with extracellular agonists, and subsequently release the contents of their storage granules, termed alpha ( $\alpha$ ) and dense granules. The intracellular signals that govern platelets' responses to activation are not completely understood, although platelet shape change, involving cytoskeletal rearrangement, is a critical hallmark of activated platelets. We hypothesized that the microtubule cytoskeleton could regulate granule secretion in platelets. The aim of this study was to determine if the chemical disruption of microtubules would impair platelet granules secretion upon activation by thrombin.

**Methods:** Blood was drawn from mice by retro-orbital plexus bleeding. Platelets were isolated from whole blood by differential centrifugation; purified platelets were treated with colchicine, a microtubule-disrupting drug that binds tubulin heterodimers to inhibit microtubule polymerization. Platelets treated with either vehicle (control) or colchicine (experimental) were activated with thrombin. Flow cytometry was used to measure surface P-selectin, a marker of  $\alpha$ -granule release. The quantity of ATP release was also quantified as a measure of dense granule release. Moreover, light-transmission aggregometry was used to measure the effect of colchicine treatment on platelet aggregation.

**Results:** Surface P-selectin expression, and ATP release, was unaffected by colchicine treatment at all concentrations tested (10-100  $\mu$ M), in response to thrombin (0.05 U/mL). Moreover, aggregometry data indicate that colchicine-treated platelets have similar aggregation profiles, relative to untreated platelets.

**Conclusions:** The integrity of the microtubule cytoskeleton does not appear to directly influence platelet granule secretion upon activation by thrombin. Similarly, the microtubule cytoskeleton does not appear to directly affect platelet aggregation in response to thrombin. Further work is required to fully uncover the role of tubulin in platelet function.

**Acknowledgements:** Supported by a grant from the Canadian Institutes of Health Research (CIHR) to HK and a UBC Faculty of Dentistry Undergraduate Student Summer Research Award to QN.



## Investigating MALT1 Substrate Specificity with Different CARD-BCL10-MALT1 Complexes

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**Objectives:** Mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) is a protease that regulates immune responses. MALT1 activity is highly regulated, requiring interaction with B cell lymphoma/leukaemia 10 (BCL10) and one of four coiled-coil linked Caspase Recruitment Domain containing proteins (CARD), to form a “CBM” complex. MALT1 and BCL10 are widely expressed, but, the expression of CARD9, CARD10, CARD11, and CARD14 is cell type-specific, making CBM composition cell type-specific. It is unknown if the CARD protein affects MALT1 substrate specificity. This project aimed to determine if CBM composition affects MALT1 substrate specificity, and identify model cell lines to assay CBM activity.

**Methods:** To identify CBM-specific cleavage, plasmids encoding the CBM components for the four complexes were co-transfected with MALT1 substrates in HEK293 cells. The proteins were subsequently analysed by Western blot. These results informed the development of a bioinformatics workflow, which utilizes RNA and protein expression data to identify cell lines in which endogenous cleavage of substrates by the CBM can be detected.

**Results:** Twenty-two MALT1 substrates were screened, and three substrates (CILK1, ILDR2, and LIMA1) showed differences in cleavage between different CBM complexes. Using our bioinformatics workflow, we identified HMC-1, RPTEC/TERT1, Daudi, and T-47d cell lines as the best cell lines to investigate CARD9, CARD10, CARD11, and CARD14 CBM activity respectively.

**Conclusions:** While a minority of MALT1 substrates are preferentially cleaved by specific CBMs, CBM composition largely does not affect MALT1 substrate specificity. Our identification of cell lines that express different CBM complexes allows for greater efficiency in discovering and validating MALT1 substrates.

**Acknowledgements:** This project was completed as part of the Centre for Blood Research (CBR) Summer Studentship Program.

## Facebook Endodontic Groups as a Tool to Motivate Critical Thinking

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**Objectives:** Social networking sites are not only predominant in students' social life but are interlinked with the dentistry/endodontics curriculum. Facebook groups have been identified for their positive potential use in higher education. We hypothesized that this is also true for endodontics. There is a paucity of data on content, dynamics, and learning opportunities of endodontic study groups. This research identified some public/open Facebook endodontic groups and analyzed their posts.

**Methods:** Using a hybrid approach (quantitative-qualitative), we analyzed n=5 public endodontic groups and n=50 posts (10 from each group). A single researcher conducted data collection, and two other researchers conducted data analysis. A table aggregated information for groups (main characteristics) and for posts (diagnosis, rubber dam use, etc.).

**Results:** Groups focused on endo-clinical-cases. On average, groups had 3 posts/day, but two of the groups had 10+ posts/day. The biggest group had 198K followers. Ethical rules included "no tolerance for bullying or harassment". The posts received few likes (average=7) and usually no comments; however, the largest group had ~100 likes/post with comments. Cases were mostly non-surgical root canal treatment (75.47%) of molars (71.69%). Diagnosis was absent in 67.92% of the cases; when present, it was incomplete and/or inaccurate (AAE-classification). Sets of radiographs were complete in 58.49% of cases (with bad quality and cropped images). The rubber dam was visualized in 81.13%. Obturation was appropriate in 79.24% of cases. Coronal restoration appeared in 81.13% of cases.

**Conclusions:** Facebook endodontic groups have the potential to motivate critical thinking. Two groups had many followers and engaged dentists in case discussions; however, most discussions focused on techniques and materials, but not on diagnosis, biology and patient's wellbeing. Diagnosis was mostly absent; when present, it was incomplete and/or inaccurate. Appealing obturations were seen, but, contrastingly, pre-operative tooth information, patient's history, and diagnosis were missing.

## Examining How CSF1R+ Cell Depletion Impacts Nerve and Musculoskeletal Development

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**Objectives:** Disrupting colony-stimulating factor-1 receptor (CSF1R) activity—which is essential for macrophage and osteoclast proliferation, differentiation, and survival—results in malformation of the skull and teeth in adult mice. We hypothesize that disrupting CSF1R activity during gestation will impact nerve and musculoskeletal development in mouse embryos. The aim of this study was to investigate and define any nerve and/or musculoskeletal phenotypes that result from depleting CSF1R+ cells during gestation using the pharmacological inhibitor PLX5622.

**Methods:** To explore the contribution of CSF1R+ cells to nerve and musculoskeletal development, pregnant CD1 and C57BL/6 mice were fed the CSF1R inhibitor PLX5622 across gestation. Embryonic day 11.5 (E11.5), E12.5, and E13.5 embryos and postnatal day 1 (P1) pups were collected from mice fed control or PLX5622-laced chow during gestation. Whole-mount immunostaining was performed on E11.5, E12.5, and E13.5 embryos to visualize developing nerves using a 2H3 anti-neurofilament antibody or developing muscles using an MF20 antibody. P1 pups were stained with Alcian blue and Alizarin red to visualize cartilage and bone, respectively. Brightfield images of craniofacial and limb nerves, muscles, and skeletal structures were collected for analysis.

**Results:** We observed noticeable nerve and muscle phenotypes in PLX5622 treated embryos at E11.5. Our findings showed a shortened branching of the facial nerve and less developed maxillary nerves and limb nerves. Shortened forelimb muscle fibers were also observed. PLX5622 exposed pups displayed skeletal phenotypes, including a dome-shaped cranial vault with wide patent sutures and disruptions in ear bone ossification. Shorter limbs and changes in ossification of the hind-foot bone were also observed.

**Conclusions:** Our findings suggest that CSF1R+ macrophages and osteoclasts play important roles during nerve and musculoskeletal development. These preliminary findings serve as a foundation for future investigation into the broader range of nerve and/or musculoskeletal phenotypes that may arise as a result of CSF1R dysfunction.

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## **Intraoral Scanner Accuracy for Subgingival Finish Line with Gingival Retraction**

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**Objectives:** To evaluate the accuracy of an intraoral scanner (TRIOS3 by 3Shape) in capturing subgingival finish lines with differing horizontal and gingival displacements, and with and without retraction cords. The secondary objective is to evaluate if this would influence accuracy of the CAD crown design process.

**Methods:** A 3D-printed resin dental model was fabricated with a removable zirconia milled die of prepared tooth #46. Six groups were fabricated from removable 3D-printed resin gingiva with varying horizontal gingival displacement (0.12 mm, 0.25 mm, 0.45 mm) and 2 subgingival finish line positions of 0.5 mm and 1 mm. An intraoral scanner (TRIOS3; 3shape) was used to scan each group (18 scan per group) and the STL files were superimposed with a reference scan obtained by scanning the die separately using the intraoral scanner. Absolute marginal discrepancies were measured on 8 fixed points among each group. A one-way ANOVA test was used to measure statistical differences between the 6 groups and a post hoc test was used to compare each group.

**Results:** All groups showed increased marginal discrepancies on the buccal, distal, and mesial surfaces. Group 1, with 0.12 mm horizontal displacement and 0.5 mm subgingival finish line, showed an average marginal discrepancy on the buccal and distal area of more than 100 microns (121 and 105 microns, respectively).

**Conclusions:** Within the limitation of this *in vitro* study and with ideal clinical settings in subgingival finish lines, the intraoral scanner shows greater accuracy with at least 0.25 mm horizontal displacement of the gingiva. Increased inaccuracy is expected on the proximal margins independent of the gingival horizontal displacement.

## **Near-Infrared Imaging Effectively Screens Proximal Enamel Caries: Diagnostic Accuracy Study**

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**Objectives:** To evaluate the diagnostic accuracy of near infra-red imaging (NIRI) technology of iTero Element 5D intra-oral scanners (index test) and compare it to bitewing radiographs (clinical reference standard) for detecting proximal enamel caries (target condition).

**Methods:** We conducted a diagnostic study on 42 consecutive patients (22 males: 20 females) who presented for orthodontic treatment at the University of British Columbia (UBC). Consenting individuals  $\geq 12$  years of age with moderate/high caries risk received intra-oral digital scanning using iTero Element 5D scanners and full-mouth bitewing radiographs (BWRs). Two pediatric dentists independently evaluated the non-restored proximal surfaces on BWRs and NIRI images for the presence/absence of initial enamel caries.

**Results:** The prevalence of the target condition in the sample population was 5.8%. Of 1110 proximal surfaces included in the analysis, the diagnosis of the target condition was confirmed in 65 surfaces based on BWRs. The index test showed a sensitivity of 60.0%, specificity of 97.7%, positive predictive value of 61.9%, and negative predictive value of 97.5%. The inter- and intra-examiner reliability for BWRs and NIRI images were 0.78, 0.97, 0.85, and 1.0, respectively. The Area Under the Curve (AUC) value for the index test was 0.78, which can be considered acceptable in terms of the test's ability to diagnose surfaces without the target condition.

**Conclusions:** The index test showed high specificity and negative predictive value; therefore, it has potential as a non-ionizing screening tool for the detection of proximal enamel caries in permanent teeth, particularly in adolescents with a moderate/high caries risk.

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## **Outcome Analysis of Endodontic Microsurgery in UBC's Graduate Endodontics Program**

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**Objectives:** Endodontic microsurgery (EMS) is an essential surgical endodontic treatment modality indicated when conventional endodontic treatment and nonsurgical retreatment fails to eradicate periapical pathology or when a biopsy is required. Therefore, we hypothesized that pre-treatment and treatment factors are associated with the prognosis of apical microsurgery and the outcome predictors. The aim of this study was to assess the level of success of endodontic microsurgery (EMS) during the last 1 to 11 years in the Graduate Endodontics (GE) program at UBC, and to examine the effect of pre-treatment, treatment, and patient factors on treatment success.

**Methods:** Fifty-eight patients (92 teeth) who had EMS at the UBC GE clinic were included in this project and invited for a follow-up appointment. Clinical and radiographic examinations assessed the long-term treatment outcome and its potential determinants. Different variables were collected retrospectively and prospectively. The periapical (PA) criteria used for the radiographic healing assessment were complete, incomplete, uncertain, and unsatisfactory. The cone-beam computed tomography (CBCT) criteria have three quantitative indices and four categories: complete, incomplete, uncertain, and unsatisfactory. The statistical analysis was performed using univariate, bivariate, and logistic regression analysis to determine the prognostic variables affecting the outcome. The significance level was set at 5%.

**Results:** Using CBCT, the favourable outcomes were 84.62% and 15.38% were not favourable (including cases with vertical root fracture [VRF]). Whereas the outcomes using PA radiographs were 60.51% for complete healing, 17.95% for incomplete healing, 7.69% for uncertain healing, and 5.85% for unsatisfactory healing. Also, the interrater reliability was in almost perfect agreement, and was higher using CBCT versus PA.

**Conclusions:** The success rate of EMS was very high overall. The treatment sequence, VRF, and presence of radiographic signs of persistent apical periodontitis using CBCT were the only factors that affected the treatment outcome.

## **Pre-Implant Tracing of Mandibular Canals on CBCT Using Artificial Intelligence**

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**Objectives:** Accurate localization of mandibular canals (MC) is important in dental implantology to avoid damaging the nerve inside the canal. Artificial intelligence (AI) has impacts on improving the quality of delivering implantology. The aim of this study was to evaluate the accuracy of the AI system with conventional methods in detecting MC during pre-implant planning using Cone Beam Computed Tomography (CBCT) images.

**Methods:** A pilot study of six CBCT scans of a dentate mandible was initially conducted and indicated a statistically significant agreement ( $p < 0.5$ ) of MC localization among manual, AI, and cadaver (gold standard) measurements using the ANOVA method. Subsequently, a total of 47 patients' CBCT scans of their mandibles were analysed for MC localization by manual tracing software (CoDiagnostiX) and AI software (Diagnocat). Measurements were performed in premolar and molars areas bilaterally and coded as LM2, LM1, LP2, RP2, RM1, and RM2. Data were calibrated for inter-examiner reliability. Independent samples t-tests were used for statistical analysis.

**Results:** 110 CBCTs were screened, while 57 CBCTs were excluded due to different Regions of Interest (ROI), 2 CBCTs were excluded in the manual software due to old DICOM files, and 4 CBCTs were excluded in the AI software due to metal artifacts or extended ROIs outside the mandible area. We had 12 CBCTs in the AI software that showed incidental findings: thickening of the maxillary sinus membrane or dense bone islands in the mandible. Statistical comparisons indicated no significant difference among the measurements of the 2 methods.

**Conclusions:** AI software is as accurate as CoDiagnostiX for MC detection.

## **Minimally Invasive Endodontic Protocol With Multisonic Irrigation: Assessment of Outcome**

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**Objectives:** Vertical root fractures almost exclusively involve endodontically treated teeth and, as a result, the concept of minimally invasive endodontics has been proposed within the discipline. However, critics of minimally invasive endodontics believe that a conservative approach may negatively influence the outcome of root canal treatment. The aim of this study was to assess the treatment outcome of a new endodontic treatment protocol, which encompasses minimally instrumented canals, a multisonic irrigation system, and single cone obturation technique with bioceramics sealers. We hypothesize that there is a difference in the treatment outcomes between the Multisonic Irrigation Protocol and conventional endodontic treatment.

**Methods:** 120 teeth were treated by two different endodontists in the province of British Columbia. Patients were treated using a standardized treatment protocol consisting of instrumentation to a maximum apical diameter of #20 without orifice enlarging, the GentleWave™ procedure, and single cone obturation with bioceramics sealer. Clinical signs and radiographic assessments were evaluated at 12-36 months to assess the treatment outcome. Periapical radiographs were assessed by two independent calibrated examiners and the periapical index score was used to score and compare preoperative and postoperative results. Success was classified as healed or healing and accounted for the cumulative success rate of healing. Statistical analyses were performed by using the Fisher's exact test, Pearson correlation, and multivariate logistic regression analysis.

**Results:** 114 out of 120 teeth were seen for recall, which is a 95% recall rate. The overall success rate across both groups was determined to be 82%. The success rate for the GentleWave™ group was higher than the success rate with conventional therapy. Apical surgery was required more often post conventional endodontic treatment than when GentleWave™ was used.

**Conclusions:** GentleWave™ has been able to adequately clean minimally instrumented canals, and the rate of apical surgery seems to have declined as well.



## **Impact of COVID-19 on Achieving Required Competencies in Orthodontic Programs**

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**Objectives:** COVID-19 invoked many restrictions on graduate orthodontic programs. This may have impacted the development of required competencies for the graduation of orthodontic residents. We hypothesized orthodontic graduates in the COVID-19-era (2020-2022) feel less competent as compared to pre-COVID-19 graduates (2019). The aims were: to develop a list of competencies for orthodontic graduates, use this list to assess perceived competencies, and identify common challenges among orthodontic programs due to COVID-19 and how these challenges were overcome.

**Methods:** An orthodontic competency list was based on available information from Canada, the USA, and Europe. Part A of the Qualtrics® survey included information about the perceived competencies of orthodontic graduates (2019-2022) from accredited Canadian and American programs. Graduates were also asked about the importance of achieving these competencies and the best modes of learning for them. Part B asked Program Directors/faculty from each program to identify COVID-19-imposed challenges in achieving specific competencies and the accommodations made to overcome them.

**Results:** Pre-COVID-19 graduates (2019) had higher levels of perceived competencies compared to COVID-19 graduates (2020-2022), and significant differences were found for 8 competencies. There were no significant differences in perceived competencies between Canadian and American graduates. Major COVID-19 imposed challenges identified by the faculty included having fewer patients/patient visits/less of a variety of malocclusions to treat and less instructor feedback. Few significant differences were observed between Canadian and American programs. Limited accommodations were needed to overcome COVID-19-imposed challenges, with the most common needs being to introduce additional clinics/seminars, reducing aerosols, and teleorthodontics.

**Conclusions:** Restrictions on orthodontic education due to COVID-19 impacted the perceived competencies of graduates, where pre-COVID-19 graduates felt more competent than graduates during COVID-19. There were no significant differences between Canadian and American graduates. Graduate programs identified a few COVID-19-imposed challenges in achieving competencies; however, few accommodations were needed to overcome them.

## Fatigue Resistance of XP-3D Shaper in a Dynamic Condition

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**Objectives:** The purpose of this study was to evaluate the effect of canal curvature locations, rotational speeds, and vertical stroke lengths on the cyclic fatigue resistance of XP-3D Shaper (XPS) files in customized artificial canals at body temperature with dynamic motion incorporated.

**Methods:** One-hundred and twenty XPS (size 30/.01) instruments were subjected to cyclic fatigue testing inside tapered artificial canals with a tip diameter of 0.40 mm, curvature of 60°, 3-mm radius, and two different curvature locations with the centers of curvature placed at 5 and 8 mm from the coronal opening. The experiment was video recorded, and each instrument was rotated by an 8:1 reduction handpiece connected to a ProMark torque-controlled endodontic motor with a defined in-and-out axial movement until a fracture was observed visibly. The speed of rotation was set at 1000 or 3000 rpm by the electric motor and repetitive axial movement of 3 or 6 mm was performed by a Hydraulic Instron Universal testing machine at a speed of 1 mm/second. The time to fracture was measured in seconds, and the number of cycles to failure was calculated. The length of the fractured instrument was also evaluated. Data were analyzed using an independent samples t-test and logistic regression at a 5% significance level.

**Results:** XPS tested with higher rpm had a lower Number of Cycles to Failure (NCF) than files tested at lower rpm. XPS with 6-mm length vertical strokes led to a higher NCF compared to when files were moved with 3 mm vertical up-and-down motions. The canal curvature location had no effect on the NCF of XPS files.

**Conclusions:** Within the limitation of the study, a long vertical stroke and lower rpm setting for XPS may reduce the likelihood of file separation. Clinicians may consider using XPS regardless of the location of canal curvature.

## Extra-Oral Bitewings in Proximal Caries Diagnosis in High-Caries-Risk Children

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**Objectives:** Although conventional intra-oral radiography (mainly bitewings and periapicals) has been central to the diagnosis of caries for the last century, recently this has been refuted in two major studies, which instead found the visual-tactile clinical examination superior. Not only does conventional radiography impart radiation, which is particularly important in association with increased radiation sensitivity in children, but it is also an aerosol-generating procedure. If radiation is indeed required, then amidst this ever-evolving pandemic extra-oral bitewing (EBW) radiographs may be preferable in a pediatric setting for caries diagnosis. To date, the sole studies have been *in vitro*, and though encouraging they indicate a higher likelihood for false positives. The aim of this study was to evaluate the false positive rate and false negative rate of EBW radiographs when compared to clinical dental examinations, which are the superior strategy for caries diagnosis. This is the first study in which findings on EBW radiographs were compared with a clinical visual-tactile examination.

**Methods:** A retrospective study of EBW radiographs in the diagnosis of proximal caries in children with a high caries risk was performed. The treatment notes of 194 patients treated at a private pediatric dental office were reviewed to identify false positive and false negative rates. Treatment plans based on EBW radiographs were compared to the treatment executed. Analysis was done by two independent and calibrated examiners.

**Results:** A total of 194 treatment records were analyzed, the EBW radiograph false positive rate was 1.8% and the false negative rate was 0.8%. Carious lesions that contributed to the false positives were incipient and lesions that the practitioner wanted to “watch” rather than treat.

**Conclusions:** Extra-oral bitewing radiographs are a promising addition to the available caries diagnostic tools, particularly during this COVID-19 pandemic, whereas the intra-oral bitewing (IBW) is an aerosol-generating procedure.

## Treatment Outcomes of Invisalign First System

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**Objectives:** The Invisalign First system was introduced to treat malocclusions in the mixed dentition in 2018. Currently, few studies are available regarding its treatment efficacy and predictability of results. Knowing the success rate of different orthodontic treatment modalities and their limitations is critically important for the success of an orthodontist as a clinician and for patient welfare. The objective of this study was to determine whether the final achieved tooth movements are coherent with Invisalign First clincheck predictions in mixed dentition.

**Methods:** This retrospective study consisted of 41 patients, 20 females and 21 males, treated with the Invisalign First system (Align Technology Inc., Tempe, USA). The mean age was 8.9 year and ranged from 6.9 to 11.2, while the average treatment time was 8 months. Pre-treatment, post-treatment achieved results, and predicted clincheck digital models were superimposed and measured for overjet, overbite, intercanine width, intermolar width, and six types of tooth movements (mesiodistal inclination, mesiodistal rotation, mesiodistal angulation, buccolingual translation, mesiodistal translation, extrusion, intrusion). Three statistical tests (Wilcoxin signed rank test, t-test, and sign test) were performed to check if achieved and predicted results were the same.

**Results:** For t-tests, overbite, intercanine/intermolar width, molar inclination, and extrusion/intrusion had  $p < 0.05$ , thereby rejecting the null hypothesis. The results of the paired t-test, when compared to the Wilcoxin signed test, were in perfect agreement and the 95% confidence intervals were very similar for the above-mentioned variables. The sign test confirmed that predicted values for overbite, intercanine/intermolar width, molar inclination, and molar extrusion/intrusion were greater than achieved values. However, overjet, molar rotation, molar angulation, and molar mesiodistal/buccolingual translation in achieved results are close to what was predicted in clincheck.

**Conclusions:** With the Invisalign First system, clincheck overestimates the predicted results vs what we achieved. Over-correction might be needed when using this system in mixed dentitions.

**Acknowledgements:** Drs. Abdulrahim Alwafi, Nancy Heckman, and the Department of Statistics, University of British Columbia.

## **Marginal Fit and Internal Adaptation of 3D-Printed and Milled Crowns.**

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**Objectives:** To compare the marginal fit and internal adaptation of 3D-printed ceramic filled hybrid composite definitive crowns and milled monolithic zirconia milled crowns.

**Methods:** A single die STL file was used to create a congruent base with six points of reference using Ivoclar's CAM5 software and milled. Fourteen samples were manufactured for each group (n=14): milled zirconia (MI) and printed (PR). The e.max ZirCAD crowns were milled and sintered using a fast-sintering protocol. The VarseoSmile Crown Plus crowns were printed and post-processed with the SprintRay Pro 95 3D printer system. All crowns were 'cemented' using ultra-light body PVS to replicate the misfit space under a standard load. A dual scan protocol was used to digitize the misfit space. Geomagic Control X 2020 was used to assess the misfit of the marginal gap (MG), occlusal surface (OC), and axial surface (AX) of each sample in both groups. IBM SPSS Statistics was used to calculate the means and standard deviations of the internal (AX and OC) and marginal discrepancies (MG) for each group. Since the data was not normally distributed and Levenè's test was significant, a non-parametric Mann-Whitney test was used to analyze the data.

**Results:** The marginal discrepancy was larger in the MI group as compared to the PR group, but the difference was not found to be statistically significant ( $p>0.05$ ). The discrepancy in the internal adaptation at AX and OC were found to be larger in the PR group compared to the MI group and the difference was statistically significant for both AX and OC measurements.

**Conclusions:** 3D-printed VarseoSmile Crown Plus crowns have similar marginal fit when compared to milled e.max ZirCAD zirconia crowns. However, the milled zirconia crowns appear to have improved internal adaptation when compared to the 3D-printed crowns.

**Acknowledgements:** Angus Barrie, BC Digital Dental Studio

## **Novel Method to Integrate Intraoral Scans with 3D Facial Images**

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**Objectives:** 3D imaging is becoming more mainstream with advances in digital technology and reductions in cost. 2D imaging has been the standard in orthodontics but it would be beneficial to examine virtual 3D patients for diagnosis, treatment planning, assessment of growth, and treatment outcomes without ionizing radiation use. This study aimed to validate Bellus Arc7 3D scanners and test a novel method for integrating intraoral scans with 3D facial images to create virtual 3D patients.

**Methods:** Part I entailed validation of Arc7 using the previously validated 3dMD face system as a reference. Three subjects (2 humans, 1 mannequin) were selected, and for each four images were taken on two occasions, one week apart, and repeated twice. Images were superimposed and a 3D heat map was generated for comparison. Part II—comparing the novel merging method—included a sample of 15 participants recruited from UBC’s Graduate Orthodontic Program. For each subject, five images were captured, with two images using a Trios intraoral scanner: one scan of the upper teeth and palate and one of buccal surfaces of the upper anterior teeth, nose, and forehead. Three 3D facial images were captured using Artec space spider (reference) and Arc7. The data was analyzed using Geomagic Control X processing software to generate a 3D heat map.

**Results:** Part I showed the root mean square for Arc7 compared to the reference 3dMD was below 2 mm for all three subjects, which is clinically acceptable for soft tissue measurements. Part II showed that integrating intraoral scans with 3D facial images to create a virtual 3D patient is possible with this novel method, albeit very technique sensitive.

**Conclusions:** The results indicated that 3D facial images acquired with Arc7 are sufficient for most clinical applications, including creating virtual 3D patients by merging intraoral scans with 3D facial scans.

## Calcified Carotid Artery Atheroma on Dental Radiographs: a Retrospective Study

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**Objectives:** Calcified carotid artery atheroma (CCAA) can be identified incidentally on dental panoramic radiographs (DPRs). What is the prevalence of CCAA detected on DPR's of individuals over 30 years old?

**Methods:** In this retrospective study, we screened 700 consecutive subjects aged  $\geq 30$  with a DPR from October 2018–April 2021 from UBC's Dental Clinic. Patient charts were reviewed for existing statin-indicated conditions as outlined by the CCS 2021 Guidelines. Prevalence of CCAA and periodontal bone loss on DPRs was assessed and associated with pre-existing conditions.

**Results:** Out of the 700 consecutive subjects with a DPR, 657 were unique. Sixteen were excluded due to undocumented medical history (45.1% females and 54.9% males). Mean age was  $60.4 \pm 16.0$  years. Only 358 (55.9%) were diagnostic for evaluation. There were 82 (22.9%) who had evidence of suspected CCAA on DPRs. The prevalence of CCAAs in females was 48% while in males it was 52%. Only 6 (7%) were identified by student dentist! Periodontal bone loss was observed in 45 (24%) of the positive CCAA. Out of those with CCAAs, 17 (20.7%) were already on lipid-lowering therapy for a pre-existing condition and 15 (18.3%) were not on lipid-lowering therapy but had a pre-existing condition. CCAA on DPRs constituted a new diagnosis for atherosclerosis for 62% of subjects. With chi-square tests, statistically significant association of CCAA was found with increasing age ( $p < 0.05$ ), male sex ( $p < 0.05$ ), pre-existing conditions ( $p < 0.05$ ), and hypertension ( $p < 0.05$ ).

**Conclusions:** DPRs can identify patients with untreated atherosclerosis. On chest CT scans, calcified coronary arteries are regarded as established coronary artery disease. Through this study, DMD students are encouraged to actively look for calcifications on DPRs. This project will lay the foundation for formalized shared care pathways between dentists and physicians, especially for patients who are  $< 50$  years old and do not see their MD annually.

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## Teledentistry Content in Canadian Dental and Dental Hygiene Schools' Curricula

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**Objectives:** Despite teledentistry's (TD) expanded utilization, it has yet to be fully adopted by oral health care professionals. The present study undertook a scoping review to explore TD incorporation in the training of oral health care providers and a survey at Canadian dental and dental hygiene schools.

**Methods:** The Joanna Briggs Institute's methodology was used for the scoping review performed by two reviewers. Studies published between 1989 and June 4, 2022, were searched. An anonymous survey with 37 questions was distributed among all 10 dental and 35 dental hygiene schools across Canada. The survey focused mostly on TD teaching (methods employed, content taught, and barriers to implementing TD), with descriptive and inferential data analyzed using SPSS®.

**Results:** A total of 2180 documents were found; 19 were eligible for data extraction. Didactic education and hands-on practice were the most employed training methods. All of the dental (n=10) and 68% (n=24) of dental hygiene schools responded to the survey; of the 34 programs, 18 had TD content, including three dental schools. An average of  $9.22 \pm 4.86$  hours was reported for teaching TD, with lecture format the most employed approach and using TD in dental practice the most covered topic among 11+ others listed. While 53% of the dental hygiene programs employed formative and summative assessments, only one dental school reported having an assessment for this content. Moreover, programs that dedicated less than 9 hours to this content were less likely to address more than seven topics (OR=0.14).

**Conclusions:** There is variation in the existing TD educational programs, from content to assessment, in both the scoping review and survey. Less than half of the dental hygiene and 30% of the dental programs incorporated TD content. However, the sparsity of TD educational programs emphasizes the necessity for its future planning.

**Acknowledgments:** The authors would like to thank all the respondents from the dental and dental hygiene schools across Canada for their participation in the TD content survey, and we would also like to thank UBC's Faculty of Dentistry for providing a token of appreciation to motivate participation.



## Silanization Kinetics: Effects of Silicon Dioxide Content and Contact Time

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**Objectives:** Silanization is essential to improve bonding to silica-based substrates. This study evaluated the effect of the substrate silica content and application time of an experimental silane solution on the kinetics of silanization, as determined by contact angle.

**Methods:** A 3% 3-methacryloxypropyltrimethoxysilane in ethanol solution was prepared and monitored by Fourier transform infrared spectrophotometry. Borosilicate glass (70-87% silicon dioxide) and quartz (99.9% silicon dioxide) with 15 mm diameter were ground using 120- and 320-grit SiC paper. For each glass, 11 groups (n=10) were created: control; etched with 9.5% HF for 30 s; and after etching and application of 5  $\mu$ L silane solution for 10 s, 20 s, 30 s, 1 min, 2 min, 5 min, 10 min, 30 min, and 60 min. The reaction was interrupted at each time with water rinsing (30s), followed by ultrasonication in water (2 min) and air drying (30s). Contact angle was assessed after each treatment. Data was analyzed by two-way ANOVA and Tukey HSD ( $p<0.05$ ) using SPSS.

**Results:** Si-OH groups in silane solutions are stable after 5 h activation. Silanization is dependent on contact time of silane with the surface and on the silicon dioxide content of the glass. Both untreated glasses presented similar contact angles that were significantly reduced after etching ( $p<0.05$ ). At 2 min, the contact angle became significantly higher than in the control for both glasses ( $p<0.05$ ). A significant increase of contact angle occurred between 2-10 min and the effects of silicon dioxide content became evident as the contact angle for quartz glass increased significantly higher than for borosilicate glass ( $p<0.05$ ). Highest contact angles were observed for both glasses after 10-30 minutes of application, with quartz glass presenting significantly higher contact angles ( $p<0.05$ ).

**Conclusions:** Silanization is more effective with increased application time and dependent on the silica content of the substrate.

## Antimicrobial Potential of Gold Nanoshells Loaded on Experimental Dental Resins

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**Objectives:** Secondary caries are reported to be responsible for a great percentage of the restorative procedures performed in dental clinics. This study explored the antibacterial properties of a methacrylate-based dental resin loaded with gold nanoshells (AuNPs) in conjunction with photothermal therapy (PTT).

**Methods:** First, 20h minimum inhibitory concentration (MIC) assays (n=6) were performed on irradiated solutions of AuNPs (120 nm, Nanocomposix; 200 to 0 µg/mL) with planktonic *Streptococcus mutans* in BHI. Light treatments were: dark; 15min LED (660nm, 11.1 J/cm<sup>2</sup>); 30min LED (660nm, 22.2 J/cm<sup>2</sup>); and 30s laser+2mm distance (95.49J/cm<sup>2</sup>). Next, experimental resin disks containing 0, 1×10<sup>10</sup>, or 2×10<sup>10</sup> AuNPs/ml were fabricated and the degree of conversion (DC) after 740mW/cm<sup>2</sup> light curing was measured using FTIR (PerkinElmer, Spectrum Two, USA). Lastly, colony forming unit (CFU) assays (n=9) were performed following 24h growth of a *S. mutans* biofilm on 6mm-diameter resin disks. Light treatment groups for the CFU assays were: dark, 30min LED (22.2 J/cm<sup>2</sup>), 60min LED (44.4 J/cm<sup>2</sup>), 30sec laser+2mm distance (95.49 J/cm<sup>2</sup>), and 30sec laser+4mm distance (23.87 J/cm<sup>2</sup>). ANOVA testing (SPSS, α=0.05) was used to analyze results.

**Results:** The MIC results showed MIC<sub>50</sub> and MIC<sub>90</sub> to be at 25 (P=0.008) and 50µg/ml (P<0.001). Additionally, 30min LED light and laser illumination showed a reduction in bacteria presence compared to dark and 15min LED (P<0.001). The DC results showed no significant differences from the control. The CFU results showed no significant dependence on AuNP concentration following any observed light treatment.

**Conclusions:** In this study, the AuNPs expressed antibacterial effects against planktonic *S. mutans* but not in a polymerized dental adhesive resin following PTT. The DC was not affected in the presence of AuNPs. We recommend future studies to investigate novel nanoparticles, including different shapes and sizes of AuNPs, to promote oral health and prevention.

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## **Barriers and Facilitators to Vaccination among People with Altered Immunocompetence**

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**Objectives:** Individuals with immune deficiency are at increased risk of morbidity and mortality caused by vaccine-preventable diseases. Despite the advantages of vaccination, some members of this high-risk population question or refuse vaccines. This study aimed to identify the barriers and facilitators to vaccination among people with altered immunocompetence via a scoping review and survey.

**Methods:** A scoping review using the Joanna Briggs Institute’s framework identified relevant publications that reported factors affecting vaccine behaviours among the immunocompromised population. Studies published from 1974 until July 25, 2022, were searched using keywords such as “vaccination”, “vaccine”, “confidence”, “hesitancy”, “uptake”, and “immunocompromised” in databases including MEDLINE (Ovid), CINAHL, EMBASE, Web of Science, PsycINFO, Cochrane Database of Systematic Reviews, ProQuest, TRIP, CHODARR, OpenGrey, and Google Scholar. Relevant peer-reviewed and grey literature, regardless of their language, were added. An anonymous 12-item survey was distributed among people living with HIV in Vancouver as a pilot study to explore vaccine behaviours and factors which deter or promote vaccine uptake.

**Results:** Overall, 3788 titles and abstracts were screened against the inclusion criteria by two reviewers, and 203 references were obtained for a full-text screening. Out of 80 participants, 91.8% were vaccinated with at least one dose of the COVID-19 vaccine, while 6.9% received a booster dose. The unvaccinated respondents were most concerned about the long-term side effects of the vaccine (57.1%) and contracting COVID-19 (42.9%). The primary barrier was inadequate information about the vaccine (57.1%), while the main facilitators of vaccine uptake were hopes of protecting themselves (80.8%), their community (60.3%), and their loved ones (57.5%).

**Conclusions:** The barriers to vaccination, including long-term safety concerns and lack of adequate information, are amendable. Advocating for policies to mitigate these barriers could reduce vaccine hesitancy and ultimately improve the uptake of vaccines among people with altered immunocompetence.

**Acknowledgements:** The authors acknowledge collaboration with the AIDS Vancouver staff in creating and distributing the surveys, the community members who participated in the study, and The Dr. Peter Centre/AIDS Foundation for funding the study.

## Detecting DNA Aneuploidy in Fanconi Anemia with DNA Image Cytometry

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**Objectives:** Individuals with Fanconi anemia (FA) have up to a 700-fold elevated risk of developing oral squamous cell carcinoma compared to the general population, resulting in a higher incidence of mortality and morbidity. Biopsies have traditionally been the only way to diagnose oral lesions. However, biopsies are not always feasible as the disease can cause frequent and multiple lesions. This investigation aimed to assess the potential of DNA image cytometry (DNA-ICM) to diagnose oral cancer by measuring aneuploidy in FA patients with oral cancer.

**Methods:** Forty-two brushings were collected from 15 FA patients with oral lesions. For each lesion, the brushing was first used for conventional cytology then DNA-ICM analysis was performed. Papanicolaou stained cytology slides were de-stained and re-stained using the Feulgen-Thionin stain (a DNA stoichiometric stain), then scanned using the MoticEasyScan™ scanner. Their proprietary software MotiCyte measures the cell nuclei's DNA content and automatically classifies them as lymphocytes, leukocytes, or epithelial cells. DNA index was calculated for each cell using the predominant normal epithelial cell subpopulation DNA as a diploid reference.

**Results:** This preliminary study shows that 45% of the samples had low cellularity (around 500 cells), which resulted in inconclusive data. This low cellularity could result from collection error or loss of cells after re-staining, thus, limiting the detection of DNA aneuploidy in FA patients. Thirty-eight percent of samples had at least one aneuploid cell, whereas 88% contained tetraploid or cycling cells. There was strong agreement between our cytometry findings and the conventional cytology conducted by the cytopathologist.

**Conclusions:** DNA-ICM can be a non-invasive, high-throughput tool to identify lesions at high risk of transformation, triage lesions, and spare low-risk lesions from unnecessary biopsies. However, further optimization of DNA-ICM of FA samples in this study is needed.

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## Potent Inhibitor of Cathepsins as Potential Skin Anti-Aging Agent

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**Objectives:** Skin protects the organs from environmental exposure. Its molecular components such as collagen and elastin are synthesized by skin-resident fibroblasts and keratinocytes and are responsible for the skin's strength and elasticity. Skin aging is a complex process caused by intrinsic and extrinsic factors. Intrinsic proteolytic degradation of the collagen-elastin matrix by skin-resident proteases (cathepsin K, V, and S), together with extrinsic photoaging, lead to skin-aging visible as wrinkling. The aim of the current study is to identify a cathepsin inhibitor and an inhibitor-delivery formulation to prevent skin deterioration.

**Methods:** We screened 31 tanshinones for elastin degradation in an *in vitro* assay for Cat K, V, and S. In addition, we harvested skin tissues from 3-month-old mice and incubated these samples with the most effective tanshinones, Odanacatib (against Cat K) and E-64 (against most cysteine proteases), and compared their effects on histological skin changes after 1-month incubation with skin samples in the absence of inhibitors. We also optimized the polymer drug-delivery formulations for these inhibitors for the better penetration into skin for future *in vivo* experiments.

**Results:** About 30% of the tested tanshinones prevented elastin degradation by inhibiting all or individual cathepsins. We also observed a clear difference in terms of extracellular matrix preservation in skin sections incubated with and without inhibitors. In a step towards *in vivo* experiments, we evaluated different encapsulation methods for one of the tanshinones, T06, and the specific CatK inhibitor, ODN, to standardize their encapsulation efficacy and drug release capability and managed to encapsulate up to 80% of inhibitors with 24-hour release.

**Conclusions:** The inhibitory activity of these inhibitors against Cat K, V, and S constitutes an interesting therapeutic potential against cathepsins-driven elastin depletion occurring during skin aging. Simultaneously, optimized drug carriers will help to deliver inhibitors deeper into dermis to minimize proteolytic damage.

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## **A National Investigation of Barriers to Pursuing Further Education**

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**Objectives:** Canada has seen a growing movement towards developing new pathways to further dental hygienists' education, strengthen cognitive/practice outcomes, and increase career pathways. According to the 2021 Canadian Dental Hygienists' Association (CDHA) Job Market and Employment Survey, only 27% of respondents earned a bachelor's degree. This study examined levels of interest and barriers to pursuing further education among Canadian diploma-holding dental hygienists. Research question: what are the levels of interest and barriers, if any, to pursuing further education amongst Canadian dental hygienists holding a diploma as their highest educational credential?

**Methods:** E-focus groups were first conducted to inform the construction of an online national survey, distributed to CDHA members. The survey collected demographic information, levels of interest, and barriers to pursuing further education. Qualitative data using Saldaña's approach of assigning descriptive and *in vivo* coding and quantitative data was analyzed using SPSS version 28.0 software. Ethics approval was granted by the University of British Columbia's Behavioural Research Ethics Board (H20-00455).

**Results:** Of 918 dental hygiene respondents, 553 (60%) expressed an interest in pursuing further education. Barriers included: time (56%), finances (53%), family commitments (51%), work responsibilities (48%), stress/anxiety of returning to school (40%), accessibility to education (34%), unfamiliarity with existing programs (34%), not being aware of eligibility (32%), competitive application process (27%), employer support (25%), family support (18%), completing online courses/using technology is intimidating (18%), and lack of reliable internet service (13%). Levels of interest and barriers to pursuing further education were compared with survey demographic data to identify associations. A majority of respondents (92%) indicated perceived benefits to pursuing further education.

**Conclusions:** Barriers were identified in both the e-focus groups and the online survey. National and provincial associations and educational institutions can use the results to help reduce barriers and raise awareness of post-diploma education for Canadian dental hygienists.

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## **First-Year UBC DMD Students' Attitudes and Behaviours Toward Support Resources**

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**Objectives:** This study investigated attitudes, knowledge, and behaviours of first-year Doctor of Dental Medicine (DMD) students toward existing student support resources within the University of British Columbia (UBC)'s Faculty of Dentistry (FoD), as well as the resources needed from the student perspective to inform development of student support programming.

**Methods:** An online survey was distributed to all 59 first-year DMD students in April 2022. This survey included three parts: 1) demographics, 2) Likert-scale questions inquiring about the perception and utilization of available support resources, and 3) open-ended questions exploring academic/non-academic experiences. A 5-student focus group followed (May 2022) to further explore themes emerging from the survey.

**Results:** Forty-six students (RR=78%) participated in the survey. Quantitative analysis revealed that most participants (70%) were familiar with FoD support resources, but only a quarter (24%) have accessed them. Few (26%) were familiar with resources across UBC, and most (98%) have not accessed them. Some (26%) felt stigma prevented them from accessing resources across campus, including within the faculty. More respondents who identified with she/her pronouns knew who to contact for academic support in comparison to respondents who identified with he/him pronouns (70% vs. 53%;  $P=0.029$ ). No statistical significance was found between age and utilization of resources ( $P=0.071-0.720$ ). Themes that emerged from qualitative analysis included: challenges with transitioning into a professional program, imposter syndrome, stigmatization of accessing resources, suggestions for increasing mentorship with senior DMD students, and unfamiliarity with existing resources. Focus group respondents elaborated on their experiences and challenges, including recommendations to further support student needs.

**Conclusions:** Grounded in the student voice, this study informs future support programming and development of student support systems at UBC and other institutions. Recommendations include improving awareness of the availability and purpose of support resources within the FoD and peer mentorship opportunities with senior DMD students.

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## **Application of the Life Grid in Oral Health Research**

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**Objectives:** Life grid tools have been used in interview-based retrospective studies to improve recall, decrease bias, and facilitate rapport concerning life events. This review explores the use of life grids specifically in oral health research and its applications.

**Methods:** This scoping review employed the Joanna Briggs Institute framework, with two reviewers screening the literature independently. Studies published until April 21, 2022, were searched using “life grid” and “oral” as initial keywords. The searched databases included: Medline (Ovid), CINAHL, PsycInfo, ERIC, MedEdPortal, Web of Science Core Collection, ProQuest Dissertations and Theses Global, and Google Scholar. All relevant literature, regardless of language, were added, and a comprehensive table was used for data extraction focused on seven main areas. The results are presented using a narrative summary.

**Results:** A total of 724 records were identified. After duplicates were eliminated, 624 records underwent title and abstract screening; 46 were selected for full-text review. A total of 22 were included for data extraction. Of the 13 presenting their application methods, eight used the life grid at the beginning, four used it during the interview, and one at the end. Also, life grids were used with participants of various ages and in different forms ranging from a flip chart with a timeline, to a table with 12 columns of categories. Minimizing recall bias and enhancing data reliability were the most frequently mentioned advantages in oral health research, mostly concerning oral cancer. The grid also helped establish rapport with the participants and allowed for checking the accuracy of the information provided.

**Conclusions:** Life grids have been applied with wide flexibility in structure and application, with researchers and participants showing positive perspectives on their application. This information can help design future oral health research and dental education, considering the life grid as a data collection and interview tool.



## Metagenome Sequencing of Oral Biofilms after Exposure to Antimicrobial Agents

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**Objectives:** To assess the long-term effect of the antimicrobial peptide DJK-5 and chlorhexidine (CHX) on oral biofilm recovery in different nutritional conditions, and identify residual and recovered bacterial biofilms using DNA sequencing technology. We hypothesized that the microbial composition of residual or recovered biofilms differ from untreated biofilms and is affected by the treatment type and nutrition availability.

**Methods:** Oral biofilms were grown anaerobically on collagen-coated hydroxyapatite discs in 24-well plates containing cultural brain heart infusion (BHI) broth for 3 weeks. A total of 192 biofilm samples were prepared and treated with either 10 µg/ml DJK-5 peptide, 2% chlorhexidine, or sterile water. Out of the 192 samples, 12 were used for immediate baseline assessment, while the rest were returned to the 24-well plates and submitted to anaerobic incubation at 37°C for use in assessing biofilm recovery over time. The BHI was changed weekly for half of the samples (refreshed BHI group), while it was left unchanged for the other half (unrefreshed BHI group). For biofilm recovery assessments, samples were collected and frozen at 1, 3, 8, 12, 16, and 20 weeks following the baseline treatment. After collecting all samples, DNA extraction was performed and the 16S rRNA gene from each sample was sequenced using third-generation sequencing technology.

**Results:** Regardless of the treatment applied, there was a significant difference between refreshed-BHI groups and non-refreshed-BHI groups in terms of the diversity of residual species. There were also changes in the diversity of the biofilm as it was recovering over time. Moreover, some species were more abundant in the biofilms exposed to CHX than the ones exposed to peptide DJK-5, and vice versa.

**Conclusions:** Species richness and diversity of residual and recovered oral biofilms depend on different factors. These factors are the type of antimicrobial treatment, time, and availability of culture media.

## Worsening Oral Health-Related Quality of Life from Childhood to Adolescence

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**Objectives:** To measure the impact of individual characteristics (e.g., demographic, socioeconomic, behavioural, and oral health factors) on oral health-related quality of life (OHRQoL) from childhood to adolescence. Individual characteristics in early childhood were hypothesized to worsen OHRQoL over time.

**Methods:** A prospective cohort study followed a sample of 639 children (T1 baseline at 1-5 years old) in southern Brazil for 10 years, with three subsequent reassessments at T2, T3, and T4. OHRQoL was measured through the Early Childhood Oral Health Impact Scale (ECOHIS) and Child Perceptions Questionnaire (CPQ8-10 and CPQ11-14) according to the age group; standardized scores ranged from 0 (better OHRQoL) to 100 (worse OHRQoL). Demographic (sex), socioeconomic (skin color and household income), behavioural (dental attendance, reason for dental attendance, and frequency of toothbrushing), and oral health measures (dental caries - ICDAS) were collected at T1 only. Multilevel Poisson regression analysis was conducted.

**Results:** A total of 469, 449, and 429 children were re-evaluated at T2, T3, and T4 (response rates of 73.4%, 70.3%, and 67.1%, respectively). The overall OHRQoL scores showed an increase over time (T1=4.61; T2=6.49; T3=10.57; T4=20.51), which indicates a worsening in quality of life. Children from low-income families, who had not been to the dentist in the last 6 months, and who brushed their teeth less than twice a day at T1 were at greater risk of worsening OHRQoL over time. In addition, children who went to the dentist for non-routine reasons and who had dental caries also exhibited a higher risk of worsening OHRQoL.

**Conclusions:** Socioeconomic, behavioural, and oral health-related characteristics established in early childhood can influence the worsening of OHRQoL during the transition to adolescence. Although some characteristics are unchangeable (e.g., sex and skin color), others including income and oral hygiene practices can be modified to decrease inequalities in OHRQoL.

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## **Visualizing Epithelial Rearrangements During Avian Lip Fusion in Real-time**

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**Objectives:** Understanding craniofacial development is a prerequisite to preventing serious congenital malformations such as orofacial clefting. The most common subtype is non-syndromic cleft lip with or without cleft palate (NSCL/P), which occurs in 1:700 live births. Typical cleft lip is unilateral rather than symmetrically bilateral, suggesting that local environmental effects as opposed to genetics are a major cause. Our objective is to image the face and to test the role of cell rearrangements in the lip fusion process.

**Methods:** Face organ cultures from E4.5 chicken embryos were incubated in a stain which fluorescently labels polymerized/filamentous actin (F-actin). The organ cultures were then transferred to 0.6% agarose/live media mix in multichambered slides. Time-lapse imaging was carried out with an inverted confocal microscope in an environmental chamber. Images were captured every 10 minutes for 5-8 hours. Cell rosettes and vertices were identified using the Morphological Segmentation plugin in ImageJ and tracked over time to identify patterns in cellular rearrangements.

**Results:** Scans of both the surface epithelia and the fusion zone between the frontonasal mass and maxillary prominences showed hexagonal cells arranged in rosettes. Over time, the rosettes lost their organization around a central cell and became elongated. In addition, the epithelial cells developed processes that budded off fragments of cell membrane. At deeper levels, epithelial cells flattened and merged in areas of contact in the fusion zone. The elongation of multi-cellular rosette formations, as well as the reduction in width of the epithelial seam, suggests that cellular intercalation is a major mechanism during fusion.

**Conclusions:** The dynamic changes in epithelial structure near the fusion zone identifies key areas of rearrangement that may be susceptible to environmental perturbations. Future studies will investigate the regional effects of inhibition of actinomyosin contractility on lip fusion.

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## Oral Health Sciences Students' Understanding of Wellbeing: An Exploratory Study

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**Objectives:** Wellbeing is a complex and multi-faceted concept that has recently gained popularity in oral health sciences education. Maintaining students' wellbeing is essential for their academic performance and overall quality of life. While many definitions and frameworks of wellbeing exist, their applicability to oral health sciences education environments remains to be elucidated. This qualitative study aimed to assess the applicability of the Feeney and Collins's framework of wellbeing to oral health sciences education by exploring the perceptions and experiences of oral health sciences students in the University of British Columbia regarding wellbeing.

**Methods:** An Interpretive Description (ID) methodology was employed in the study design, data collection, and data analysis. Semi-structured interviews were used to collect data from dental and dental hygiene students. Interviews were transcribed verbatim and imported into NVivo 12. Transcripts were coded and analyzed with guidance from Feeney and Collins' wellbeing and thriving framework. Categories and sub-categories were inductively developed within and beyond the organizing categories of the chosen framework.

**Results:** Thirty-one oral health sciences students participated in the study. Study data largely fit into the six wellbeing domains suggested by Feeney and Collins: physical, psychological, eudaimonic, subjective, and social. Spirituality emerged as a new wellbeing domain. Inter-domain relationships were observed. The social domain seemed to contribute to all other wellbeing domains, while the subjective domain seems to be shaped by all the other domains.

**Conclusions:** The development of an extended version of the Feeney and Collins' framework seems to be useful to understand and conceptualize wellbeing in oral health sciences education. Further evidence is needed to demonstrate the instrumental value of the identified domains and their corresponding dimensions.

## Evaluation of SARS-CoV-2 Proteases and Its Variants

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**Objectives:** SARS-CoV-2 produces 16 non-structural proteins (NSPs) which underwent numerous mutations in the known viral variants. From these NSPs, homo-dimeric 3CL-M<sub>pro</sub> liberates 12 proteins from the poly-protein, and the papain-like protease (PL<sub>pro</sub> domain in NSP3) liberates 4 NSPs. To date, three major mutant variants are known for 3CL-M<sub>pro</sub> (Beta VoC; K90R), (Lambda VoC; G15S), and (Omicron VoC; P132H) but none for PL<sub>pro</sub>. Because of their essential importance in the viral life cycle, studying the viral proteases and their variants can be beneficial to understand viral pathogenicity. The understanding of their activities will define them as antiviral targets for the development of more effective inhibitors.

**Methods:** Recombinant 3CL-M<sub>pro</sub> variants and PL<sub>pro</sub> were purified from *E. coli*, and characterized using fluorescence-based substrates, by thermal stability assays. Thirty-one *Salvia miltiorrhiza*-derived tanshinones were evaluated for their inhibitory activity on wild-type 3CL-M<sub>pro</sub> and the most potent compounds was screened for all variants. Molecular docking was used to predict potential binding sites. Nirmatrelvir, the 3CL-M<sub>pro</sub> inhibitor, was tested against WT and mutant variants. The characterization of PL<sub>pro</sub> is presently ongoing.

**Results:** WT and three mutants had comparable kinetic parameters and pH profiles. Stability assays showed that 3CL-M<sub>pro</sub> from Omicron was slightly less stable than others. Out of 31 tanshinones, one has shown inhibition for all variants. Molecular docking predicted active site and distal binding for this inhibitor in the vicinity of the dimerization site. Nirmatrelvir proved active against the WT enzyme and its variants.

**Conclusions:** Characterizing 3CL-M<sub>pro</sub> variants showed comparable substrate kinetics, but slightly different stability data. Moreover, selected tanshinones have the potential to inhibit 3CL-M<sub>pro</sub> of SARS-CoV-2 and to act as potential antivirals. They may provide a platform for future medicinal chemistry efforts for viral protease inhibitors. Current mutations in 3CL-M<sub>pro</sub> have not yet affected the potency of the antiviral Paxlovid in *in vitro* assays.

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# Applications, Functions, and Accuracy of Artificial Intelligence in Restorative Dentistry

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**Objectives:** Applications of artificial intelligence (AI) are increasing in dentistry. With studies suggesting the improved functionality of AI models in various aspects of restorative treatments, we hypothesized that AI models in restorative dentistry are promising compared with traditional methods in terms of accuracy. The aim of this literature review was to comprehensively assess the reported applications, functions, and accuracy of AI in restorative dentistry.

**Methods:** A literature search was performed using the key terms of artificial intelligence; machine, deep, or computational learning; artificial or convolutional neural network; clustering; soft computing; automated planning or reasoning; and computer vision both separately and jointly. In addition to a manual search, an electronic search of Medline/PubMed, Embase, Web of Science, Cochrane, Scopus, and Google Scholar was conducted to identify related original or review, English-language articles published between January 1, 2000 and March 1, 2022, relevant to restorative dentistry.

**Results:** After applying inclusion/exclusion criteria to 525 initially-identified articles, 157 articles were included, reviewed, and discussed regarding applications, functions, and accuracy of AI models. Articles were categorized into 7 categories: caries detection, tooth preparation, margin detection, tooth restoration design, metal structure casting, dental restoration/implant detection, removable partial denture design, and tooth shade determination. A wide range of accuracy levels are reported in each category, with variations depending upon the type of AI model, the source and size of the training data, the method of verification, and the control methods/groups. Efforts are being made to gather more training data, with an aim to enhance the AI models' performance.

**Conclusions:** AI models in restorative dentistry provide promising objectivity compared with traditional methods in regards to decision-making for clinical outcomes in many of the aforementioned categories. Future studies are required to validate their accuracy and apply them to routine dental practice.

## Bioinformatic Prediction and Experimental Validation of New MALT1 Substrates

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**Objectives:** Mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) is a master regulator of NF- $\kappa$ B signaling in immune responses. MALT1 first amplifies, but later attenuates, NF- $\kappa$ B by sequential proteolytic cleavage of negative and positive regulators of the pathway. A reduction in MALT1 expression and/or activity is associated with numerous pathologies, including oral lesions and oral carcinoma. However, clear insight into the role of MALT1 in disease is hampered by incomplete knowledge of the full repertoire of MALT1 substrates. The goal of this study was to identify new MALT1 substrates.

**Methods:** We created an algorithm to predict MALT1 substrates. Our algorithm integrates knowledge of protein function and sequences to rank MALT1 substrate candidates by similarity to known substrates. We tested the ranked predictions by co-transfection of candidates with constitutively active MALT1, and monitored substrate cleavage by Western blot. Using catalytically inactive MALT1 (Cys464Ala), a MALT1 inhibitor (MLT-748), and noncleavable P<sub>1</sub>-Arg to Ala mutant versions of each substrate in dual transfections, we validated the new substrates *in vitro*.

**Results:** We identified seven new MALT1 substrates in the co-transfection screen: TANK, TAB3, CASP10, ZC3H12D, ZC3H12B, CILK1, and ILDR2. We confirmed the cleavage of endogenously expressed TANK and the RNase ZC3H12D in B cells by Western blotting and mining of proteomics datasets.

**Conclusions:** The new substrates that we discovered open new frontiers for exploration of MALT1 function within and beyond oral biology. Furthermore, our strategy of integrating protein function with sequence information improves MALT1 substrate prediction precision. Thus, a similar approach could be utilised to improve existing algorithms designed to predict the substrates of other proteases.

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