



17TH ANNUAL  
**PACIFIC**  
RESEARCH DAY

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# Abstracts

Faculty, Student and Staff Presentations

First & Second-Year Student Research Presentations

Senior Research Presentations

IDS Student Review Presentations

Stockton Campus Student Presentations

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# **FACULTY, STUDENT AND STAFF PRESENTATIONS**

# **A practical comparison of exosome isolation kits for primary endothelial and monocytic thp-1 cell samples with low working volumes**

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**OBJECTIVES:** Use of proprietary exosome isolation kits may be driven by lack of an ultracentrifuge, limited sample volume, high sample number or even unfamiliarity with the field of exosome research. Using commonly available commercial kits we examined the relative recovery of product from low volume samples of cell culture supernatant media from primary human endothelial cells (HEC) and monocytic THP-1 cells. Samples of 4ml and under are realistic in vitro working volumes and are within the advertised ranges of all kits, indeed 4ml is the maximum suggested volume for one tested kit.

**METHODS:** Cells were grown to subconfluency then washed and exposed to serum-free media for 48 hrs. Conditioned media was collected by low speed centrifugation, cell number and viability was determined. To standardize samples, media from several flasks was pooled for each cell line. Samples were processed according to manufacturer guidelines. Relative recovery was determined by protein content, using a standard colorimetric assay. Protein content is a primary sample determinant for downstream studies such as western blot. T-test was performed, n=5 for all samples except THP-1 Pure-Exo which was n=4.

**RESULTS:** Mean sample protein content was highest for the Invitrogen kit in both HEC, 11.3 µg/sample and THP-1 cells, 26.28 µg/sample. Recovery from each kit was significantly different from the others ( $p \leq 0.005$ ) for both HEC and THP-1, following the order Total Exosome Isolation (Invitrogen) > Exoquick (System Biosciences) > PureExo (101Bio). Notably mean total recovered protein from a 4ml sample was mostly lower than the normal usable limit for downstream protein assays such as western blot. Recovery values were in a similar range to samples prepared by differential ultracentrifugation. To compare relative recoveries from both cell lines, protein mass per million cells was calculated; HEC sample levels were markedly higher than THP-1 levels.

**CONCLUSIONS:** The decision to use a commercial kit for exosome recovery may have many influences but low protein recoveries for all kits - from sample volumes common to cell culture studies - lends caution to that choice if protein expression analysis is the intended end-point.

*This work was supported by Research Pilot Project Award DR03-102 from the Arthur A. Dugoni School of Dentistry, and presented at the International Society of Extracellular Vesicles 2015 Annual Meeting, Washington.*

## **Program Relocation for Superior Interprofessional Education Opportunities, Improved Financial Strength and Increased Capacity for Patient-Centered Care**

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**Background, Challenge or Opportunity:** Since 2002, the University of the Pacific's baccalaureate Dental Hygiene program has operated on the main university campus, 90 miles and 1 ½ hours away from the School of Dentistry. As one of only 56 bachelor degree dental hygiene programs nationally, Pacific's program prepares graduates for positions as clinicians, educators, researchers, public health and industry professionals. The School of Dentistry moved to a new facility in 2014, presenting an opportunity to co-locate the dental hygiene (DH) program with dental (DDS), and international dental studies (IDS) programs currently located there. Evidence suggests that by offering DH, DDS and IDS students the opportunity to learn and practice collaboratively, patient care will be improved and practice-readiness enhanced. Administrative support for the DH program will additionally be customized and strengthened.

**Purpose/Objectives:** The purpose of this project is to transition the school's DH program from its current satellite location on the main university campus in Stockton, CA, to the dental school's new facility in San Francisco, CA. Accomplishing this move by January 1, 2017, with matriculation of DH2018, will allow the school to:

Improve quality of interprofessional teaching, learning and patient care for DH, DDS, IDS programs; streamline financial and administrative infrastructure for improved efficiency, effectiveness, sustainability, and responsiveness to DH student needs; and increase the quality and capacity for clinical patient services via co-therapy

**Methods/Approach:** A task force was created and charged by the Dean. The following transition considerations were examined: strategic plan congruence, admissions policies and support, student financial aid, registrar services, curricular changes, patient care, physical space considerations, financial impact, and effect on community stakeholders. Assumptions, recommendations and limitations were generated and iteratively reviewed by the task force and stakeholders. A final transition proposal was developed and submitted to the Dean, with subsequent acceptance by University leadership in late 2014.

**Outcomes and Evaluation Strategy:** Operational plans are being developed for final move completion in June 2017. Transition success will be evaluated through assessment of DH program applicant numbers, quality and yield; DH student success on standardized board and licensure examinations; graduate satisfaction and alumni participation metrics currently in use; capacity and delivery of clinical services and patient satisfaction. Achievement of financial benchmarks outlined in the proposal will be monitored. Finally, a comparison of DH, DDS and IDS attitudes regarding interprofessional collaboration, pre and post move, will be examined using a modified version of the Jefferson School of Attitudes Toward Physician-Nurse Collaboration survey.

## **Comparison of the cutting efficiency of instruments manufactured with conventional nickel-titanium and novel gold metallurgy in lateral action**

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**OBJECTIVES:** The purpose of the present study was to compare lateral cutting efficacy of two different rotary systems manufactured with either conventional nickel-titanium or novel gold alloy, ProTaper Universal (PTU) and ProTaper Gold (PTG) (Dentsply Tulsa Dental Specialties).

**METHODS:** Seventy-eight PTU and PTG Shaping Files instruments (SX, S1 and S2), with the same exact geometries but manufactured with two different alloys were used for this study. An Instron machine was used to test bending behavior for each instrument in triplicate to determine the ideal displacement to generate a standardized force of 2N against the substrate. Ten instruments of each size were used in simulated lateral action during 60 seconds (s) against an acrylic substrate engaged in a computer-controlled testing platform at 300rpm. The process was repeated four times producing 4 notches in each plastic block after 60s, 120s, 180s and 240s action. Notch areas and depths were measured under a stereomicroscope, and Student T test was used to compare data between alloys. Repeated measures ANOVA, was used to compare cutting efficiency for each instrument across the four different time points.

**RESULTS:** For all three Shaping Files (SX, S1 and S2) PTG instruments were significantly more cutting efficient ( $p < 0.05$ ) at the four different times point, except for S1 that did not show significant differences at 240s time point. No significant differences were found in cutting efficiency for any specific instrument after the four different time points.

**CONCLUSIONS:** ProTaper Gold showed higher cutting efficiency in lateral action probably due to the advancements in the proprietary metallurgy after area and length analysis of notches produced in a plastic substrate. None of the instruments showed a significant loss in cutting efficacy over the 4mins of testing.

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## **RANK/RANKL – OPG Pathway System**

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**INTRODUCTION AND OBJECTIVE:** Orthodontic tooth movement is based on site-specific bone remodeling that is induced by therapeutic mechanical stresses applied to teeth. Bone resorption occurs on the compressed side, whereas bone formation occurs on the stretched side of the periodontal ligament space and alveolar bone. It is the aim of this literature review to give the reader an overview of cellular and molecular mechanisms involved in orthodontic tooth movement.

**METHODS:** Relevant recent articles were reviewed and the new information was combined with the previous knowledge.

**RESULTS:** The mechanical force loaded onto a tooth affects the periodontal ligament first. The cells within the periodontal ligament respond to the mechanical stress by secretion of molecules that can induce resorption or formation of bone. Osteoclasts accumulate on the compressed side of an orthodontically moving tooth and resorb the alveolar bone. Receptor activator of nuclear factor kappa B (RANK) is a membrane bound protein, expressed on surface of cells belonging to the monocyte-macrophage lineage. Osteoclastogenesis is mainly regulated by two cytokines, receptor activator of nuclear factor kappa B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF). RANKL is produced by osteoblasts. Binding of RANKL to RANK starts differentiation of monocytic progenitors into functional osteoclasts in the bone tissue. Osteoprotegerin (OPG) is a decoy receptor for RANKL. It is also secreted by osteoblasts and competes for RANKL. A proper balance between RANKL and OPG determines, if bone is resorbed or formed.

Current exploration of this system has led to an increased understanding of a potential of various therapeutic modes. Locally administered bioactive molecules were shown to stimulate or inhibit tooth movement via manipulation of the RANK/RANKL- OPG pathway system. A potential of gene therapy targeting this system was studied in respect to improving results of bone grafting and of treatment of bone metabolic diseases, such as osteoporosis and arthritis. Finally, research on low-energy laser irradiation suggested that it may enhance velocity of tooth movement via induction of RANK and RANKL.

**CONCLUSIONS:** The knowledge of how the RANK/RANKL–OPG pathway system works can have a significant impact on current and future treatment modalities within the fields of dentistry, especially benefiting orthodontic patients.

*This review project was a part of the seminar series at the Research Fellowship Program supported by the Department of Orthodontics, University of the Pacific, Arthur A. Dugoni School of Dentistry*



## **Interleukin-1beta as biomarker of periodontal reaction in teeth moved by Invisalign**

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**INTRODUCTION:** Chewing of food requires not only a stability of teeth, but also a plasticity of their position in the alveolar bone. Both functions are provided by periodontal ligament (PDL). Application of orthodontic force on a tooth leads to injury of PDL that reacts by a sterile inflammatory reaction. One of the earliest detectable biomarkers is interleukin-1beta (IL-1b). The aim of our study was to determine, if changes of IL-1 $\beta$  concentration could be detected in the gingival crevicular fluid (GCF) of the teeth moved by Invisalign.

**METHODS:** GCF was collected from twenty teeth at baseline before treatment, on the 1st and the 14th day of the first aligner, on the 14th day of the third aligner, on the 1st and the 14th day of the fifth aligner wear. Periostrips (Oraflow) were used for collection of GCF and a volume of GCF was measured using Periotron (Oraflow). IL-1  $\beta$  concentrations were measured by ELISA (Ray Biotech). The baseline value was subtracted from the values measured during treatment. Significance of differences between means was determined using paired t-test.

**RESULTS:** In our preliminary study, we found that concentration of IL-1b was increased on the first day and decreased towards the 14th day of the first aligner wear.

**CONCLUSION:** It seems that IL-1  $\beta$  can be used as early biomarker of PDL reaction in biomechanical studies of teeth moved by Invisalign.

## **Gingival Crevicular Fluid Volume in Patients Treated With Invisalign**

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**INTRODUCTION:** Invisalign system has a firm place among techniques of orthodontic tooth movement (OTM). However, very little is known about underlying cellular and biochemical mechanisms. We studied gingival crevicular fluid (GCF) volume as an early sign of inflammatory response to OTM.

**METHODS:** Our prospective study had six participants (IRB approval Nr. 14-57). We followed twenty teeth at baseline and at five time points over 14 weeks into treatment with Invisalign. Periopaper strips (Oraflow) were used for GCF collections and Periotron (Oraflow) for volume measurements. Means per groups of teeth were calculated and significance of differences was determined by paired t test.

**RESULTS:** Major increase of GCF volume occurred at the start of the first aligner. Subsequent aligners triggered a diminishing response. A GCF volume measured after two weeks of aligner wear was always lower than the value at the start.

**CONCLUSION:** GCF volume changes, when compared to baseline of the same patient, seemed to follow loading of the teeth with the aligner. The study will be continued with the focus on concentration of cytokines in GCF.

*This study was supported in part by the Research Pilot Project Award 03-Activity 094 from the Arthur A. Dugoni School of Dentistry, University of the Pacific.*

# **TransfeX and TransIT-LT1-Mediated Gene Delivery to Cervical and Oral Squamous Cell Carcinoma Cells**

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**OBJECTIVES:** Cancer gene therapy involves the introduction of therapeutic genes into malignant cells. The use of lipid-based non-viral vectors is a promising approach to gene delivery since it avoids potential immune effects presented with the use of viral vectors. However, the transfection efficiency of non-viral vectors needs to be improved to achieve adequate transgene expression in most cancer cells. In particular, oral squamous cell carcinoma cells are highly resistant to transfection by non-viral vectors. We investigated the ability of two novel reagents to deliver the gene for firefly luciferase into cervical and oral squamous cell carcinoma cells.

**METHODS:** HeLa cervical carcinoma, and HSC-3 and H357 human oral squamous cell carcinoma cells were seeded in 48-well culture plates one day prior to transfection, and used at ~85% confluence. The cells were transfected with either TransfeX (ATCC, Manassas, VA) or TransIT-LT1 (Mirus, Madison, WI). Each transfection reagent was used at ratios of 1, 2, 4, or 8  $\mu$ l per 1  $\mu$ g plasmid DNA (pCMV.Luc). Toxicities of the reagents were measured with the Alamar Blue cell viability assay. Transfection activity was evaluated by measuring luciferase expression 48 h after transfection, using the Luciferase Assay System (Promega) and a Turner Designs TD-20/20 luminometer. Data were expressed as relative light units (RLU) per ml of cell lysate.

**RESULTS:** In HeLa cells, luciferase expression with 4  $\mu$ l TransfeX/1  $\mu$ g DNA reached 50,620,000  $\pm$  13,195,000 RLU/ml, the highest activity ever seen in our laboratory. TransIT-LT1 at 4  $\mu$ l/1  $\mu$ g DNA achieved 10,221,000  $\pm$  1,616,000 RLU/ml. In HSC-3 cells, luciferase expression obtained with TransfeX was 1,035,000  $\pm$  37,000 RLU/ml, and 35,000  $\pm$  6,000 RLU/ml with TransIT-LT1. In H357 cells that are highly resistant to transfection with previously available reagents, TransfeX showed maximum transfection efficiency at 2  $\mu$ l TransfeX/1  $\mu$ g DNA, with 2,514,000  $\pm$  250,000 RLU/ml.

**CONCLUSIONS:** While TransIT-LT1 is not quite as effective, TransfeX facilitates unusually high levels of transgene expression at levels comparable to that achieved by adenoviral transduction (our unpublished data). Transfection of HeLa cells with TransfeX exhibited the highest transfection activity obtained in our laboratory, and was almost 5 times higher than that achieved with TransIT-LT1. In HSC-3 cells, transfection efficiency of TransfeX was 29 times higher than that of TransIT-LT1. Very high levels of luciferase activity were obtained in H357 cells with TransfeX. TransfeX is likely to be beneficial in various cancer gene therapy approaches and other gene therapies involving non-viral vectors.

## **Interleukin-1beta and dentistry**

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**INTRODUCTION:** Interleukin–1beta (IL-1 $\beta$ ) is a potent cytokine that enhances local inflammatory response through binding to different types of local target cells. It facilitates differentiation of monocytes into multinucleated osteoclasts and thus enhances bone resorption. Many studies concluded that IL-1 $\beta$  is a strong inducer of connective tissue degradation in general. IL-1 $\beta$  seems to play a major role in immune reactions occurring in dental and orofacial tissues.

**OBJECTIVES:** We reviewed the current knowledge related to behavior of IL-1b in oral and dental diseases and adaptations.

**METHODS:** Relevant recent articles were reviewed and the new information was combined with the previous knowledge.

**RESULTS:** IL-1 $\beta$  and periodontal disease: Studies have shown that lipopolysaccharides (LPS) present on the outer membrane of gram negative bacteria causing periodontal disease induce secretion of IL-1 $\beta$  that starts innate and adaptive inflammatory responses. Further studies have suggested that both probing depth (PD) and attachment loss (AL) are strongly correlated with IL-1 $\beta$  levels in gingival crevicular fluid (GCF). When patients with different severity degrees of periodontal disease were compared, it was found that patients with a more severe disease tend to have higher levels of IL-1 $\beta$  in GCF than those with a moderate or mild disease regardless of the PD value of the examined site.

**IL-1b and orthodontic tooth movement:** When mechanical force is applied on a tooth, a localized sterile tissue innate inflammatory response takes place. One of the first signs is secretion of IL-1 $\beta$ . Levels of IL-1b in GCF were found to peak after 24 hours of force application. A second peak of IL-1b levels was found 24 hours after re-activation of the applied force. These findings suggest that time scale of IL-1b secretion may be a good guide for timely controlled applications of force and reactivations during orthodontic treatment.

**CONCLUSION:** A better understanding of biology and physiology of IL-1b-related pathways might open the way for future improvement and/or customization of dental treatment plans.

*This review project was a part of the seminar series at the Research Fellowship Program supported by the Department of Orthodontics, University of the Pacific, Arthur A. Dugoni School of Dentistry*

## Genetics involved in palatogenesis

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**INTRODUCTION:** Mammalian palatogenesis occurs early during embryonic development. A deep knowledge of the process, by which a secondary palate is formed, is important for understanding of etiology of oral clefts, especially nonsyndromic cleft lip and palate.

**OBJECTIVES:** In this poster, we want to show new information about morphogenetic cellular and molecular mechanisms involved in palatal shelf growth, elevation, adhesion and fusion during formation of a palatal bone.

**METHODS:** Relevant recent articles were reviewed and the new information was combined with the earlier discovered information.

**RESULTS:** Important phases in formation of secondary palate:

*Palatal shelf growth:* Reciprocal epithelial-mesenchymal interactions guide the growth of palatal shelves. *Palatal shelf elevation:* Remodeling and horizontal outgrowth from the medial walls of the vertical palatal shelves provide for formation of horizontal palatal shelves. *Palatal shelf adhesion and fusion:* Mice deficient for transcription factor p63 (Tp63) and interferon regulatory factor6 (Irf6) are born with cleft palate. *Palatal Fusion:* Transforming growth factor beta (Tgfb) signaling has an important role in fusion of palatal shelves. Snail family members are also crucial regulators of palatal fusion. Apoptosis of contacting epithelia makes fusion of shelf connective tissue matrix possible. *Further growth of palatal bone:* Bmpr1a and Tbx22 transcription factors are required for palatal bone formation and growth.

Insights gained from animal models: Understanding of the genetic control of palatogenesis is mainly derived from animal experimental studies on mice, birds and zebrafish. Although many genes identified in animal models are involved in palatogenesis in humans, some mechanisms, especially those involving participation of environmental factors, are different.

**CONCLUSIONS:** Current research showed numerous factors that are essential for normal palatogenesis. If combined with results of research on environmental factors, this knowledge can be applied to development of clinical and public programs preventing development of nonsyndromic cleft lip and palate anomalies in humans.

*This review project was a part of the seminar series at the Research Fellowship Program supported by the Department of Orthodontics, University of the Pacific, Arthur A. Dugoni School*

## **Ameloblastoma: An epidemiological perspective with a focus on craniomaxillofacial reconstruction**

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**INTRODUCTION:** Ameloblastoma, belonging to the family of epithelial odontogenic tumors, accounts for 1% of tumors and cysts in the jaw and 10% of all odontogenic tumors. Ameloblastoma is a benign, slowly growing, locally expanding epithelial odontogenic neoplasm that contains ameloblasts derived from dental lamina. In the majority of cases, it occurs in the mandible. Surgery is always a radical ectomy of the tumor including almost one cm of normal bone on both sides. From a craniomaxillofacial standpoint, the goal is to restore form and function of the mandibular apparatus while reestablishing functional relationships within the oral cavity to facilitate speech and deglutination and aesthetic appearance of the face.

**OBJECTIVES:** We review innovations pertaining to in vivo engineering of bone replacement that has aroused scholarly intellects of surgeons in an effort to achieve a more functional surgical result, decrease operative burden (morbidity) and to assemble a superior three-dimensional outcome.

**METHODS:** Relevant recent articles were reviewed and the new information was combined with the previous knowledge.

**RESULTS:** A racial proclivity towards African Americans has been noteworthy in regards to the occurrence of ameloblastoma. Exceptional innovation in tissue engineering has granted reconstructive surgeons a calculable outcome to restore form and function of the mandibular apparatus.

**CONCLUSION:** Ameloblastoma affects the bones of the maxillomandibular complex, representing the odontogenic tumor of high clinical significance. A racial penchant towards African Americans, in particular African American men, has overwhelmingly been reported in the research articles that have been reviewed. As for craniomaxillofacial reconstruction, advancement pertaining to in vivo engineering of bone replacement has revealed an opportunity for surgeons to formulate a more constructive surgical result, decrease operative burden (morbidity) and to contrive a superior three-dimensional outcome in comparison to conventional reconstruction techniques.

*This review project was a part of the seminar series at the Research Fellowship Program supported by the Department of Orthodontics, University of the Pacific, Arthur A. Dugoni School of Dentistry*

## **Quail-duck chimeras reveal plasticity in signaling mechanisms dictating species-specific phenotypic features**

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**INTRODUCTION:** During evolution, differences in jaw size between vertebrate species were crucial for their adaptation to certain environments. However, extreme variation during development may cause a disease.

**OBJECTIVES:** The study in question is focused on developmental events associated with neural crest progenitors that affect differences in beak size. After the period of facial prominence formation and fusion, their further growth may differ greatly.

**METHODS:** In the mentioned study, development of duck and quail chimeras was examined. Embryogenesis: This is the time, during which organogenesis of all body systems, starting from a zygote, is finished.

**RESULTS:** Many different signaling pathways are involved that can orchestrate development of tissues and organs. The most pivotal signaling molecules are the following: (a) Sonic Hedgehog - SHH: This is a very important signaling molecule involved in organogenesis of many organs. In gene knockout mice lacking functional SHH protein, the brain, skeleton, musculature, gastrointestinal system and lungs fail to develop correctly. (b) Bone Morphogenetic Protein - BMP: Several BMP's exist in different organ systems. They were originally discovered by their ability to induce formation of bone when injected into the skeletal muscle. (c) Fibroblast Growth Factor - FGF: Growth factors of FGF family are involved in angiogenesis, wound healing and embryonic development of many organs. (d) Delta/Notch: This signaling pathway is required for regulation of cell polarity. Abnormal signaling may cause abnormal anterior-posterior polarity in somites.

**Quail-Duck Chimeras Reveal Spatio-Temporal Plasticity:** The quail duck chimeric system was used to test how dermis regulates expression of genes required for feather development. In the face and neck, the dermis arises from neural crest ectomesenchyme. Experiments showed that quail neural crest cells induced duck host epithelium to form feather buds on a quail like timetable and spatial pattern. In effect, the more quail donor cells were available in the transplanted quail dermis, the more complete was quail-like pattern of feathers in the duck host.

**CONCLUSION:** Neural crest cells regulate expression of genes, which are essential in feather morphogenesis. In particular, neural crest cells are guiding spatial and temporal patterning via regulation of signaling molecules like BMP, SHH, Delta/Notch.

*This review project was a part of the seminar series at the Research Fellowship Program supported by the Department of Orthodontics, University of the Pacific, Arthur A. Dugoni School of Dentistry*

# **1<sup>ST</sup> AND 2<sup>ND</sup> YEAR STUDENT RESEARCH PRESENTATIONS**



## **Nondestructive assessment of the remineralization of artificial enamel lesions with polarization- sensitive optical coherence tomography.**

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**OBJECTIVES:** It is difficult to completely remineralize carious lesions because diffusion into the interior of the lesion is inhibited as new mineral is deposited in the outermost layers. In previous remineralization studies employing polarization sensitive optical coherence tomography (PS-OCT), two models of remineralization were employed and in both models there was preferential deposition of mineral in the outer most layers. In this study we attempted to remineralize the entire lesion using an acidic remineralization model and demonstrate that this remineralization can be monitored using PS-OCT.

**METHODS:** Artificial lesions approximately 100–150  $\mu\text{m}$  in-depth were exposed to an acidic remineralization regimen and the integrated reflectivity from the lesions was measured before and after remineralization using PS-OCT. After sample PS-OCT imaging was completed, the samples were serial sectioned to a thickness of 200  $\mu\text{m}$  along the long axis using an Isomet 5000 saw from Buehler (Lake Bluff, IL) for polarized light microscopy (PLM) and transverse microradiography (TMR). PLM was carried out using a Meiji Techno RZT microscope from Meiji Techno Co., Ltd. (Saitama, Japan) with an integrated digital camera, EOS Digital Rebel XT from Canon Inc. (Tokyo, Japan). The sample sections were imbibed in water and examined in the bright field mode with crossed polarizers and a red I plate with 500-nm retardation. A custom built digital microradiography (TMR) system was used to measure the volume percent mineral content in the areas of demineralization on the tooth sections. High- resolution microradiographs were taken using Cu K $\alpha$  radiation from a Philips 3100 X-ray generator and a Photonics Science FDI X-ray digital imager, Microphotonics, (Allentown, PA).

**RESULTS:** Automated integration routines worked well for assessing the integrated reflectivity for the lesion areas after remineralization. Although there was a high degree of remineralization, there was still incomplete remineralization of the body of the lesion.

**CONCLUSION:** This study demonstrated that PS-OCT can be used to nondestructively measure changes in lesion structure and severity upon exposure to an acidic remineralization model. This study also demonstrated that automated algorithms can be used to measure the surface remineralization layer thickness and assess the lesion severity even with the presence of a weakly reflective surface zone.

*This work was supported by NIH/NIDCR Grant R01-DE17869.*

## Differential Regulation of C6-Ceramide-Induced Cell Death in Different Oral Squamous Cell Carcinoma Cell Lines

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**OBJECTIVES:** Survivin, a member of the inhibitor of apoptosis (IAP) protein family, is associated with malignant transformation and is over-expressed in oral squamous cell carcinoma (OSCC), but is undetectable in most normal adult tissues. C6-ceramide is a sphingolipid metabolite, with anti-proliferative and pro-apoptotic activity *in vitro* and *in vivo*. Recent studies with C6-ceramide have shown a down regulation of survivin in large granular lymphocytic leukemia. We therefore investigated the cytotoxicity and anti-survivin activity of liposomal C6-ceramide in HSC-3 and CAL-27 OSCC cells as a potential novel therapeutic agent.

**METHODS:** Liposomal C6-ceramide and control liposomes were added to HSC-3 and CAL-27 cells at 10 and 20  $\mu$ M and incubated for 24 h. Cell survival was assessed by Alamar Blue and survivin levels were measured by ELISA. The percentage of cells positive for survivin was also quantified by flow cytometry.

**RESULTS:** HSC-3 cells treated with liposomal C6-ceramide resulted in dose-dependent, decreased cell viability, measured by the Alamar Blue assay. The viability of HSC-3 cells with plain POPC:DOPE liposomes was 93% of the control. For 10  $\mu$ M liposomal C6 ceramide, the viability was reduced to 44% of untreated cells. Survivin levels in HSC-3 cells decreased from 538 pg/mg protein in controls to 310 pg/mg protein at 10  $\mu$ M C6-ceramide. However, the percentage of the HSC-3 cells positive for intracellular survivin was similar between untreated and 10  $\mu$ M C6-ceramide-treated cells. In contrast to the HSC-3 cells, CAL-27 cells were resistant to C6-ceramide-induced cell death. Basal levels of survivin in CAL-27 cells were lower than that in HSC-3 cells, and did not change significantly after C6-ceramide treatment.

**CONCLUSIONS:** HSC-3 cells are vulnerable to liposomal C6-ceramide in a dose-dependent manner, whereas CAL-27 cells are not. Liposomal C6-ceramide reduced cell proliferation in HSC-3 cells probably because of a decrease in the levels of the anti-apoptotic protein, survivin. The lower endogenous survivin levels in CAL-27 cells than in HSC-3 cells suggest that the resistance of CAL-27 cells to C6-ceramide-induced cell death may be regulated by a mechanism other than survivin.

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## Photodynamic Therapy of Oral Cancer with Liposomal Zinc Phthalocyanine

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**OBJECTIVES:** Photodynamic therapy (PDT) is a medical procedure that uses photosensitizer molecules activated with light to kill cancer cells. Liposomes are nanoparticles used for transporting the photosensitizer into the target cells. We investigated the use of zinc phthalocyanine (ZnPC) as a photosensitizer, delivered within liposomes, for PDT against CAL27 cells derived from a squamous cell carcinoma of the tongue. The effect on cell viability following irradiation was examined.

**METHODS:** Liposomes were prepared by hydration of dry lipid films in isotonic buffer, followed in some cases by extrusion through polycarbonate membranes of defined pore diameter. Two types of liposomes were prepared: palmitoyloleoyl PE (POPE) with palmitoyloleoyl PG (POPG) and dioleoylphosphatidyl ethanolamine (DOPE) with POPG. Cells incubated with extruded and nonextruded liposomal ZnPC were exposed to light (660 nm) for 30 min, and cells treated with the photosensitizer but shielded from light were used to evaluate dark toxicity. Cells incubated with medium only and free liposomes were used as controls. Cell viability was assessed using the Alamar Blue assay and a Molecular Devices microplate reader.

**RESULTS:** Extruded POPG:POPC:ZnPC liposomes irradiated with light at various delivery concentrations all showed greater reduced CAL27 cell viability compared to nonextruded liposomes irradiated with light. Extruded POPG:POPC:ZnPC liposomes irradiated with light at the highest delivery concentration of 1.0 µg/mL reduced cell viability to ~14%. Liposomal ZnPC showed no dark toxicity. Extruded and nonextruded POPG:DOPE:ZnPC liposomes irradiated with light at various delivery concentrations showed no significant changes in cell viability compared to controls.

**CONCLUSIONS:** PDT against CAL27 cells is effective using POPG:POPC:ZnPC in a dose-dependent manner, following light activation. POPG:DOPE:ZnPC does not appear to reduce CAL27 cell viability. Future studies will determine the reasons for the apparent resistance of cells to POPG:DOPE:ZnPC to PDT, the intracellular localization of liposomes labeled with fluorescent lipids, and the effect of PDT on other oral cancer cell lines such as FaDu (squamous cell carcinoma of the pharynx).

# **Torsional performance of protaper gold rotary instruments during shaping of small root canals after two different glide path preparations**

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**OBJECTIVES:** A single-instrument glide path rotary system (ProGlider) is marketed to simplify the technique of glide path preparation. There are currently no studies that assess differences in torsional performance of shaping rotary instruments after different glide path preparations. The objective of this study was to compare the torsional performance of a novel rotary system, ProTaper Gold (PTG) after two different glide path preparations in small root canals of extracted teeth.

**METHODS:** Each independent canal of eight mesial roots of mandibular molars were randomly assigned to achieve a reproducible glide path with a new set of either PathFile #1 and #2 or ProGlider after negotiation with a 10 K-file. After, glide path preparation, root canals in both groups were shaped with PTG following the same sequence. Tests were run in a standardized fashion using a torque-testing platform. Peak torque (Ncm) and force (N) was registered during the shaping procedure and were compared with Student T tests after normal distribution of data was confirmed.

**RESULTS:** One S1 instrument fractured in the PathFile group. No significant differences were found for any of the instruments in peak torque or force after glide path preparation ( $p>0.05$ ).

**CONCLUSIONS:** Under the conditions of this study, differences in the torsional performance of ProTaper Gold rotary instruments after two different glide path preparations could not be demonstrated. The different geometry of glide path rotary systems seemed to have no effect in the peak torque and force induced by PTG rotary instruments when shaping small root canals in extracted teeth.

## **The role of interleukin-17c in periodontal health and diseases: literature review and proposal of the experiment**

Cherilyn Kasperian<sup>1\*</sup>, Ryan Yu<sup>2</sup>, William Lundergan<sup>3</sup>, and Takahiro Chino<sup>2</sup>

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**OBJECTIVES:** Toll-like receptors (TLRs) expressed on oral epithelial cells (OECs) enable rapid response to microorganisms by secreting antimicrobial peptides as well as cytokines that play pivotal roles in antimicrobial immunity. Several lines of investigation have revealed that cytokines play important roles not only in tissue homeostasis, but also in the pathogenesis of infectious disease including periodontitis. OECs also express receptors for a number of different cytokines. Thus, cytokines secreted by OECs may play an autocrine role or may influence adjacent non-epithelial cells. IL-17C, a member of the IL-17 cytokine family, is mainly produced by epithelial cells. Its role in the gut-immune system has recently been proposed, however, the biological significance of IL-17C and its receptor on OECs in the maintenance of oral mucosal homeostasis and pathogenesis of periodontitis has not been systematically investigated. The purpose of our presentation is to provide: 1) current understanding of immunomodulatory roles of IL-17C in mucosal immunity, and 2) our research plan to investigate the role of IL-17C in periodontal health and disease.

### **RESULTS: Literature Review**

Colon and tracheal epithelial cells as well as epidermal keratinocytes revealed enhanced secretion of IL-17C in response to heat-killed *Escherichia coli*. Moreover, among the four TLR-ligands tested, flagellin was the most potent to induce IL-17C secretion from colon epithelial cells. IL-17C signals through the IL-17RA/IL-17RE receptor complex and acts to promote innate host defense and regulates the intestinal inflammation and barrier function. Treatment of human primary keratinocytes with graded doses of recombinant human IL-17C enhanced  $\beta$ -defensin-2 secretion while mice lacking IL-17C were partially resistant to experimental autoimmune encephalomyelitis.

### **Preliminary Data**

Our preliminary data indicated the constitutive expression of IL-17RE on human oral keratinocyte (HOK) while human gingival epithelial cell line, GSM-K cells did not express either IL-17RA or IL-17RE. In addition, HOK expressed both TLR5 and TLR9, however, GSM-K expressed only TLR9.

**CONCLUSIONS:** A literature review suggests that IL-17C has both protective and pro-inflammatory properties. Our preliminary data indicate the possible interaction between IL-17C and its receptor on OECs.

*This work will be supported by a Research Pilot Project Award from the University of the Pacific, Arthur A. Dugoni School of Dentistry (CK).*

## **Odontogenic carcinoma arising from a hybrid ameloblastoma and keratocystic odontogenic tumor: a heretofore unreported occurrence**

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Keratocystic odontogenic tumor (KOT) is the most common odontogenic tumor and is described as a cystic neoplasm with potentially aggressive local behavior. Ameloblastoma of the maxillofacial region is a locally aggressive neoplasm. Both occur predominantly in the mandible. Malignant transformation of ameloblastoma, and more rarely of a KOT has been well recognized. However, the simultaneous occurrence of these two entities as a hybrid odontogenic carcinoma of the jaws has never been documented in the literature.

This case report describes the unusual finding of a hybrid tumor showing features of both an ameloblastoma and a KOT with malignant changes in both entities.

## Virtual dental home

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In 2010, the Pacific Center for Special Care launched the Virtual Dental Home (VDH) project in California. This project uses telehealth, which requires digital technology to deliver oral health education, care, and services to increase dental care access in underserved communities. VDH utilizes a cloud-based electronic record keeping system, Denticon, while employing an on-site dental personnel such as a RDH, and an off-site dentist. The on-site RDH travels to Head Start centers or preschools to collect dental health data, which includes intraoral photos, radiographs, dental charting, and updated dental and medical histories. When they upload it to Denticon, this allows the off-site dentist to review them and conduct an examination on the respective patient. VDH has been in practice for over 5 years now and has seen over 2,800 patients with over 6,800 total visits. This study will evaluate whether geographically distributed dental providers can communicate thoroughly through an electronic cloud-based system, as well as assess whether the telehealth technology is actually playing a pivotal role in improving the dental care among underserved individuals.

## Treatment of *Porphyromonas gingivalis* with Liposome-Encapsulated Azithromycin

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**OBJECTIVES:** Periodontal diseases are being seen at an increased rate in many countries through the world. Oral antibacterial agents commonly used to treat oral bacterial infection are difficult to maintain at therapeutic concentrations in the oral cavity and can be rendered ineffective by the development of drug resistance, especially when given systemically. *Porphyromonas gingivalis* is one of the primary microorganisms responsible for the destruction of periodontal tissue. We investigated whether liposomes that bind quantitatively to *P. gingivalis* can enhance the effect of azithromycin encapsulated in the lipid phase of liposomes.

**METHODS:** *P. gingivalis* strain 2561 was grown on blood agar plates, under anaerobic conditions, scraped gently from the agar and suspended in Brain Heart Infusion media to an optical density of 0.4 at 660 nm (equivalent to  $10^8$  bacteria/ml). *P. gingivalis* was added to 96-well plates at a concentration of  $2 \times 10^5/200 \mu\text{l}$ . DOTAP:DOPE:azithromycin liposomes, DOTAP:POPC:azithromycin liposomes, free azithromycin (stock dissolved in ethanol), empty liposomes (DOPE and POPC), and ethanol alone were added to *P. gingivalis* in phosphate-buffered saline at 1  $\mu\text{g/ml}$ . After incubation for 28 h at 37°C under anaerobic conditions, the samples were read at 660 nm using a VersaMaxPLUS microplate reader.

**RESULTS:** (1) Free azithromycin at MIC (1  $\mu\text{g/mL}$ ) reduced *P. gingivalis* growth by 82.3%, based on the spectrophotometer turbidity readings. (2) Control DOTAP:DOPE liposomes reduced *P. gingivalis* growth by 12.2%. (3) Control DOTAP:POPC liposomes decreased *P. gingivalis* growth by 23.7%. (4) DOTAP:DOPE liposomes containing azithromycin had no effect on *P. gingivalis* growth in the range 0.0625 – 16  $\mu\text{g/ml}$  of azithromycin. (5) Azithromycin encapsulated in DOTAP:POPC liposomes had no effect on *P. gingivalis* growth in the range 0.0625 – 16  $\mu\text{g/ml}$  azithromycin.

**CONCLUSIONS:** Both types of liposomes were ineffective delivery systems for azithromycin. Free azithromycin was effective in inhibiting the growth of *P. gingivalis* at a MIC of 1  $\mu\text{g/ml}$ . Liposomes appear to negate the inhibitory effect of azithromycin entirely when bound to the antibiotic. A rationale for this could be that the azithromycin is bound tightly to the liposome membrane to and encapsulate the azithromycin; however, the azithromycin is never subsequently released from the liposome to act on *P. gingivalis*. From this, we can also conclude that the use of the spectrophotometer in measuring turbidity is an effective experimental technique in assessing the response of *P. gingivalis* to varying conditions.

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## **Prevalence of malocclusions among dental students**

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**INTRODUCTION:** The vast majority of studies and textbooks state that normal occlusion is observed only in 30-35% of general population. When the Angle classification of occlusion is used, the most common is the class I malocclusion.

**OBJECTIVES:** The purpose of our study was to ascertain the prevalence of malocclusions among dental students, obtain information about family history, and prepare background data for heritability estimate calculation, recurrence risk calculation, and a molecular genetic analysis.

**MATERIAL AND METHODS:** The sample consists of 284 probands (149 males, 135 females), past and present DDS & IDS dental students from the University of the Pacific Arthur A. School of Dentistry. Two forms of data collection were used: (1) A structured questionnaire for collection of descriptive epidemiologic data and (2) a family pedigree drawn by students for recording of malocclusions that occurred in the first, second, and third generation of relatives. All information was entered into Excel and descriptive statistic tool pack was used for analysis.

**RESULTS:** Out of 284 probands, 15 (5.4%) did not record their occlusion. Normal occlusion was observed only in 10.6% (n=30), Angle class I in 65.1% (n=185), Angle class II in 5.9% (n=17), and Angle class III in 7.4% (n=21). Combinations of Angle classification of the first molar on left and right side were observed in 15 cases (5.4%).

**CONCLUSION:** Our results are not based on general population data, however, the sample is large enough to provide a valuable information in respect to the prevalence of Angle type of malocclusions in a selected population. Relatively low prevalence of normal occlusion and high Angle class I group is due to inclusion of even small irregularities of line occlusion. The study continues to increase the sample size and to analyze also a family history of previous orthodontic treatments.

*Presented at the CDA Scientific Session in Anaheim, May 15, 2014.*

## **Mechanobiology and differentiation of cells**

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**INTRODUCTION:** Tooth movement and distraction osteogenesis are examples of periodontal ligament and bone adaptations to mechanical load. Does mechanobiology play a role also during ontogenetic development? In this review, we shall try to answer this question.

**METHODS:** Using PubMed, we screened the recent literature and reviewed the relevant articles that were available.

**RESULTS:** Differentiation of cells is a complex process comprising intracellular and extracellular factors and their interactions. It is a multicellular phenomenon – differentiation proceeds in groups of cells that may also interact with each other. In this review we will focus on one of the extracellular biophysical factors, namely effects of mechanical force. We will describe examples elucidating how the mechanical force is perceived and transmitted by intracellular signaling pathways to influence expression of genes in the nucleus and result in a cellular response. Biomechanical factors can even trigger some differentiation processes without participation of specific external biochemical ligands.

**CONCLUSION:** Biomechanical and biochemical factors constitute microenvironment of cells. Cellular responses to biomechanical factors belong to basic mechanisms of cellular adaptation to surrounding conditions not only during normal function after maturation, but also during ontogenetic development.

## Genetics and Class III Malocclusion

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**INTRODUCTION:** It has been known for a long time that genetics plays a role in Class III malocclusion. This condition can cause a number of problems including a compromised esthetics and difficulties in speech and mastication. Many different modes of inheritance have been investigated: autosomal dominant with or without complete penetrance, autosomal recessive, and a polygenic inheritance.

To date, several gene mutations have been found associated with Class III malocclusion, such as DUSP6, TGFB3, LTBP2, MATN1, and EBP41. They were associated solely with maxillary hypoplasia or with mandibular prognathism in specific ethnic groups. Some of them are involved in regulation of jaw growth and in cell proliferation or differentiation.

**OBJECTIVES:** The goal of our study is to determine prevalence of two genetic mutations rs20566 and rs1065755 of Matrilin-1 gene that was previously identified as candidate gene for mandibular prognathism in our sample of individuals diagnosed with Class III malocclusion.

**METHODS:** Our sample consisted of 43 individuals with Class III malocclusion and 8 of their relatives. Saliva specimens were collected from all individuals using our own protocol. Taqman genotyping (Applied Biosystems) was used to identify genotypes in two polymorphisms of the MANT1 gene: rs20566 MANT1 (+7987 G>A, Exon 5) and rs1065755 MANT1 (+8752 C>T, Exon6). Genotype proportions and allele frequencies were calculated.

**RESULTS:** As the subgroup of patients' relatives was very small (3 fathers and 4 mothers), we analyzed only patients in this study. For rs20566 MANT1 polymorphism, the highest proportion of mutated AA homozygotes was found (n=18, 41.9%), followed by AG (n=14, 32.6%), and wild type homozygotes GG (n=11, 25.3%). As expected, allele A frequency was higher (0.581) than allele G frequency (0.419). In rs1065755 MANT1 polymorphism, the highest proportion was found for wild type homozygotes CC (n=26, 60.5%), followed by CT (n=13, 30.2%), and very low mutated homozygotes TT (n=4, 9.3%). Allele C frequency was 0.756 and allele T frequency 0.244.

**CONCLUSIONS:** More data are needed to draw the final conclusions, especially whether there are differences in prevalence of these mutations between Class III and other malocclusions, and also within the Class III between familial mandibular prognathism and those cases with no family history.

## **Orthodontic and orthognathic treatment history among dental students**

Samadian Amin<sup>1\*</sup>, Sara Moazzami<sup>2</sup>, Kang Navtej<sup>2</sup>, Han Jesse<sup>3</sup>, Chandana Vundavalli<sup>2</sup>, and Marie Tolarova<sup>2</sup>

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**INTRODUCTION:** Among primary reasons why patients (or their parents) seeking orthodontic treatment is improvement in their dentofacial appearance, occlusion, the health and longevity of the dentition, and overall improvement or oral health.

**OBJECTIVES:** The purpose of our study was to find out proportion of dental students who had orthodontic treatment, considering that reasons for orthodontic treatment in our sample of dental students, were most likely prioritized more compare to general population.

**MATERIAL AND METHODS:** This study is in part continuation of our previous study of prevalence of malocclusion among dental students by Han et al (2014). We increased the sample size from original number of 284 probands to 489 (251 males, 238 females), past and present DDS & IDS dental students from the University of the Pacific Arthur A. School of Dentistry. Two forms of data collection were used: (1) A structured questionnaire for collection of descriptive epidemiologic data and (2) a family pedigree drawn by students for recording of malocclusions that occurred in the first, second, and third generation of relatives. All information was entered into Excel and descriptive statistic tool pack was used for analysis. We analyzed prevalence of malocclusions, history of orthodontic treatment and orthographic surgery, and congenitally missing teeth in permanent dentition.

**RESULTS:** Normal occlusion was observed only in 7 % (n=34) of all participants, Angle class I in 69% (n=337), Angle class II in 6% (n=29), and Angle class III in 6% (n=31). Combinations of Angle classification of the first molar on left and right side were observed in 19 cases (3.9%). In 39 (8%) probands occlusion was not clearly specified. Orthodontic treatment reported 188 (38.45%) students. The highest prevalence was observed in the Class II – out of 29 cases 19 (65.5%) had orthodontic treatment, followed by Class I – 93 cases (27.6%) out of 337, and Class III – 7 (22.6%) out of 31 had orthodontic treatment. As expected, orthognathic surgery was the highest in Class III – all but 1 (30 out of 31 students) reported having orthognathic surgery. There was no orthognathic surgery reported in subgroup of Class II students, and only 5 cases (1.5% in the Class I. The highest prevalence of congenitally missing teeth (including 3rd molars) was reported in Class II subgroup (48.3%), followed by Class III (41.94%), and the lowest (37.9%) in Class I.

**CONCLUSION:** The results of our study are not population based, however, provide us with an interesting data on history of orthodontic treatment. The sample is also ethnically diverse, which most likely is influencing results. We are continuing in increasing sample size, which will allow us to focus on specific details in more heterogeneous subgroups.

## **MSX1 in etiology of congenitally missing teeth**

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**INTRODUCTION:** A lot of research has been done on the genetic/familial aspects of dental agenesis. In particular, PAX9 and MSX1 genes seem to have a strong association with dental agenesis. Each of them codes for a transcription factor that binds to a specific region of DNA. Experimental studies on mice showed that a knockout of either gene resulted in tooth development not going past the bud stage, thus leading to a dental agenesis. In humans, MSX1 appears to take precedence over PAX9 in regards to development of second premolars and maxillary first premolars, while PAX9 appears to affect development of maxillary first and second molars and mandibular second molars.

**OBJECTIVE:** To study two polymorphisms (rs8670 C>T and rs12532 A>G) in exon2 of MSX1 gene in individuals with congenitally missing teeth previously genotyped for one mutation in exon1 and seven mutations in exon2 of PAX9 gene.

**METHODS:** Our sample consisted of 36 individuals; 24 probands with congenitally missing teeth (13 dental student volunteers and 11 patients from our orthodontic clinic), 8 mothers of probands, 2 fathers of probands, and 2 sisters of probands. Saliva specimens were collected from probands and their immediate family members. Specimens were collected using our own protocol: participants were asked to rinse their mouth with 0.5 oz of Listerine® for 30 seconds followed by a rinse with water for 30 seconds in order to get rid of food particles. They were then asked to spit into a 50 mL Falcon tube until 3-5 mL of saliva was collected. In the Craniofacial Genetics Laboratory, drops of saliva were spotted on filter paper and allowed to dry. Taqman genotyping (Applied Biosystems) was used to identify genotypes in two polymorphisms of MSX1 gene (rs8670 C>T and rs12532 A>G).

**RESULTS:** Out of 36 genetically examined individuals with hypodontia, 32 were positive for some of the MSX1 polymorphisms' mutated alleles. Only 4 individuals – 3 probands and one proband's sister had no mutated allele (they were wild allele homozygotes for both polymorphisms). We observed only one mutated homozygote TT (rs8670) in proband's father. When both polymorphisms were combined, the most common was CTAA genotype (6 cases), followed by CCAG and CCGG (5 cases each). Three individuals were heterozygotes for both polymorphisms. The numbers are not definitive, because the study is still in progress.

**CONCLUSIONS:** Results of this pilot study indicate a rather strong genetic component of hypodontia in our sample, namely a high prevalence of MSX1 gene mutations. A comparison of proportions of genotypes and allele frequencies of these polymorphisms in cases and controls will follow. Evaluation of a larger sample will enable us to draw more definitive conclusions regarding the inheritance of hypodontia related to MSX1 gene mutations and gene-gene interactions between PAX9 and MSX1.

## **A potential genetic cure for HIV: Utilizing the CRISPR/Cas9 system to cleave the HIV promoter in infected cells**

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**OBJECTIVES:** CRISPR and Cas, a system originally identified in bacteria and archaea, acts as an adaptive immune system in which CRISPR RNAs (crRNAs) and Cas proteins work together to identify and degrade complementary viral or plasmid DNA. Since its discovery, this system has been engineered for use in mammalian cells and thus became the CRISPR/Cas9 system. These systems utilize guide RNAs (gRNAs) in order to target specific sequences within genomes. We are designing CRISPR/Cas systems specific for various regions of the HIV promoter, the “long terminal repeat” (LTR). These systems, which will be part of expression vectors (plasmids), will be tested for their ability to reduce the expression of the reporter genes, luciferase and green fluorescent protein (GFP), under the control of the HIV LTR. to determine the efficacy of CRISPR/Cas9 designed to target the LTR sequence.

**HYPOTHESIS:** Successfully transfected/cotransfected cells will cause a significantly lower amount of luciferase and GFP expression than the control groups with empty CRISPR/Cas9 systems.

### **METHODS:**

Control – CRISPR/Cas9 system with no gRNA integrated

Transfect CRISPR/Cas9 System into HeLa-tat LTR-GFP cells using TransfeX

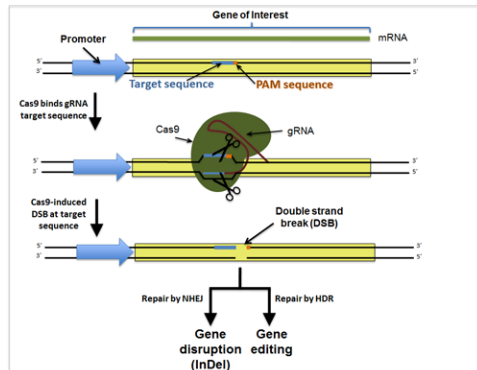
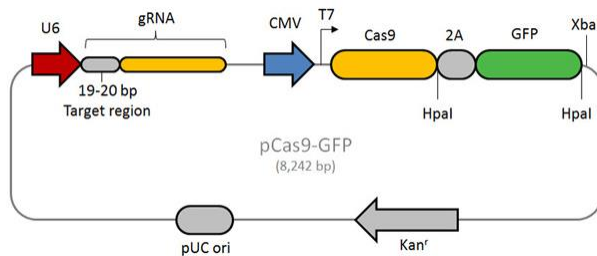
Cotransfect LTR-Luciferase and CRISPR/Cas9 System into HeLa-tat cells.

# A potential genetic cure for HIV: Utilizing the CRISPR/Cas9 system to cleave the HIV promoter in cells with the integrated HIV long terminal repeat (LTR)

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**OBJECTIVES:** Clustered regularly interspersed short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins is a system originally identified in bacteria and archaea and acts as an adaptive immune system. CRISPR RNAs (crRNAs) and Cas proteins work together to identify and degrade complementary viral or plasmid DNA. Since its discovery, this system has been engineered for use in mammalian cells and is known as the CRISPR/Cas9 system. Guide RNAs (gRNAs) in the CRISPR plasmid targets specific sequences within a genome.



Our aim is to design a CRISPR/Cas9 system specific for various regions of the HIV promoter, the “long terminal repeat” (LTR). Here, the gRNA sequence would target the HIV LTR Modulatory/Promoter/Core-enhancer regions. This construct will be designated as “pCRISPR/Cas9.LTR.”

**METHODS:** In our proof-of-concept experimental system, HeLa-Tat-III/LTR/d1EGFP cells (NIH AIDS Reagent Program) that express green fluorescent protein (GFP) under the control of LTR will be transfected with pCRISPR/Cas9.LTR, using the highly efficient TransfeX liposomal vector. Binding of gRNA to the target sequence results in the formation of a duplex and guides the double stranded cleavage of the LTR by Cas9, which is expected to cause a decrease in GFP expression due to the repair response. Controls will include a construct without the gRNA. GFP expression will be measured by flow cytometry and confocal fluorescence microscopy.

**HYPOTHESIS:** Our hypothesis is that cells successfully transfected with pCRISPR/Cas9.LTR will express a significantly lower amount of GFP than the control group with a non-targeted CRISPR/Cas9 plasmid.

# SENIOR RESEARCH PRESENTATIONS



## **Inferior alveolar nerve block injections: anatomical basis and technique simplification**

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**OBJECTIVES:** Nerve block anesthesia is commonly employed in mandibular dental procedures to serially block the inferior alveolar (IA), mylohyoid (MH), and lingual nerves. Success rates for mandibular nerve anesthesia range broadly (13-98%) depending on the patient, technique employed, and level of practitioner experience. We test a new maxillary landmark that is consistently visible in the oral cavity. This landmark does not require positional changes in the hand piece nor an arbitrary determination of insertion depth, thereby simplifying the technique.

**METHODS:** We compiled a geographically diverse skull sample ( $n=26$ ). Skulls were scanned on a GE Lightspeed VCT scanner (helical mode, 0.3mm isotropic voxels, standard convolution kernel). Triangular meshes and isosurfaces were created in Amira (v.5.4). Mandibular models were duplicated and attached with an incisal opening of ~40-45 mm. The condylar heads of one mandible were aligned on the articular eminences and the other was centered in the glenoid fossa. To mimic the injection path we oriented orthoslices through the interproximal surfaces of the LP<sub>3-4</sub> and the posterior extent of the right alveolar ridge (maxillopterygoid junction). The occlusal plane and coronoid notch were used to establish the injection height.

**RESULTS:** With the mandible at maximum opening and positioned on the articular eminence the needles terminated at the medial ramus just superior to the mandibular foramen in 73% of cases (19/26). In 7.6% of the cases (2/26), the plane ran at or just posterior to the border of the ramus. In 19.2% of the cases (5/26), the plane ran posterior to the ramus. Geographic variation is not a factor in the observed difference. The observed relationship was not affected by the presence or absence of the M<sup>3</sup>/<sub>3</sub>'s nor when the complete dentition was missing.

**CONCLUSIONS:** Assessment of the maxillopterygoid junction as a suitable landmark for needle insertion shows it to be appropriate. It also demonstrates that this landmark has the potential to greatly simplify the technique by only requiring the needle to be inserted to the depth of the mandible or ~5 mm from the hub. However, in ~20% of cases the landmark results in a slightly more posterior needle position than what at first appeared to be acceptable. However, assessment of needle insertion depths for both short and long needles showed this concern to be unfounded. Long needles terminate well before the posterior mandible when a 5 mm buffer is retained between the mucosa and hub. Use of the new landmark should result, minimally, in success rates similar to the standard and Gow-Gates techniques while simplifying the process of blocking the inferior alveolar nerve.

*A preliminary version of this work was presented at the Hinman Research Symposium, Memphis, TN 2014; The San Francisco Dental Society, San Francisco, CA 2014; and the AADR San Francisco Section Research Symposium, San Francisco, CA 2015.*

## **Osteoarthritis of the temporomandibular joint: a case study**

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*Arthur A. Dugoni School of Dentistry, University of the Pacific, Dental Practice, San Francisco, CA <sup>3</sup>. Arthur A. Dugoni School of Dentistry, University of the Pacific, Biomedical Sciences, San Francisco, CA*

### **Temporomandibular joint disorder (TMD) can have multifactorial etiology, both traumatic and atraumatic.**

**OBJECTIVE:** Determine the etiology of a complex TMD case in a 28y.o. caucasian female patient, presenting with a history of abnormal late growth, possible trauma, bilateral sagittal split orthognathic surgery, unilateral osteoarthritis, masseter atrophy, and chronic pain.

**METHODS:** Comparatively analyzed panoramic films (patient ages 8, 13, 16, 21, 24, 26), MRIs (age 21, 24), and CBCTs (age 24, 26). Reviewed orthognathic surgery notes from a bilateral sagittal split (age 22). Blood tests and neurological exams show no significant findings.

Conducted clinical consultations with orofacial pain specialists, orthodontists, and anatomists in effort to determine etiology and diagnosis.

**RESULTS:** Resorptive changes in the right TMJ became radiographically apparent at age 21; arthritic changes are first evident at age 24 (six months post-surgically).

Patient experiences chronic pain, isolated to the right side, with loci in the preauricular region, temple, and mental foramen. Patient reports constant pain of 3-4 on the pain scale, peaking at 7-8 after a long day of speaking and functioning.

The patient's initial occlusal contact in centric relation is between teeth #8 and #25. With effort, light posterior contact can be forced between #3 and #30. Dentition shows little to no signs of wear. Study models show that the teeth have not shifted significantly since surgery, suggesting post-surgical relapse.

**CONCLUSIONS:** The patient has multifactorial contribution to her condition. Her late growth caused a hyperplastic left ramus and condyle, creating a malocclusion that necessitated surgical correction. Orthognathic surgery torques the temporomandibular joint (TMJ); this torquing may precipitate or exacerbate an osteoarthritic response.

Post-surgical relapse and resorptive TMJ changes may both contribute to the patient's malocclusion. Occluding solely on incisors increases pressure in the TMJ space. This, combined with arthritic/osteophytic changes, contributes to the patient's chronic pain.

## Salivary flow rate in a rat model of type 2 diabetes

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**OBJECTIVES:** Xerostomia is usually considered a common oral complication of type 2 diabetes. Because saliva plays an integral role in oral health by helping maintain the integrity of hard and soft tissues, the purpose of this study was to examine submandibular salivary flow rate in a rat model of type 2 diabetes (Zucker Diabetic Fatty, ZDF).

**METHODS:** Male and female diabetic (ZDF obese, N=32) and non-diabetic (ZDF lean, N=33) rats were used in this study. One to 2 months after the development of diabetes, animals were anesthetized and submandibular flow was measured in response to either sympathetic (2 and 4 Hz continuously, or 20 and 40 Hz in bursts of 1s every 10s) or parasympathetic (1 to 20 Hz) stimulation. Submandibular saliva was collected and weighed, and flow rate was expressed as  $\mu\text{l}/\text{min}/\text{g}$  tissue. Differences were analyzed for statistical significance using student's t test.

**RESULTS:** Serum glucose levels were elevated in both male ( $443 \pm 96$  mg/dl vs  $144 \pm 46$  mg/dl) and female ( $437 \pm 100$  mg/dl vs  $131 \pm 24$  mg/dl) rats. Only minor differences were observed between male and female, control and diabetic rats at all frequencies of parasympathetic stimulation. However, when sympathetic stimulation was applied, significant differences in flow rate ( $p < 0.01$ ) were observed between males and females, irrespective of diabetes. Diabetes resulted in a reduction in salivary flow rate ( $p < 0.01$ ) by approximately 40% in male and 30% in female rats.

**CONCLUSION:** Only small effects of type 2 diabetes were observed during parasympathetic stimulation. In contrast, submandibular salivary flow rates evoked by sympathetic stimulation were significantly lower in male compared to female non-diabetic rats, and diabetes resulted in significant reductions in salivary flow rates in both sexes. These data suggest that type 2 diabetes may be responsible for the xerostomia often observed in diabetic patients.

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## **Introduction of extracellular vesicles into dentistry**

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**OBJECTIVES:** Extracellular vesicles (EVs) are small, membrane-enclosed entities released from cells in many different biological systems. These vesicles play an important role in cellular communication by virtue of their protein, RNA, and lipid content, which can be transferred among cells. Peripheral blood is a rich source of circulating EVs, which are easily accessible through a blood sample. An analysis of EVs in peripheral blood could provide access to unparalleled amounts of biomarkers of great diagnostic and prognostic value. The objectives of this review are to briefly present the current knowledge about Extracellular vesicles, how it can be used in dentistry, a toolbox of techniques to isolate and its clinical applications.

**METHODS:** Several techniques exist to characterize the different features of EVs, including size, enumeration, RNA cargo, and protein phenotype. Each technique has a number of advantages and pitfalls. The technique used to isolate Extracellular vesicles in this review was traditional Centrifugation method. Along with that, a comprehensive review of the literature was completed with a focus on evidence-based research articles. A hand search of relevant dental journals was also completed.

**RESULTS:** Exosomes are known to be both beneficial as well as pathologic. Initially they were characterized as a means of maturing reticulocyte to get rid of the redundant proteins but now it has been proven that they are involved with exchange of materials between cells, intercellular communications, transmission of pathogens, altering the immune system, antigen presentation, along with elimination of unnecessary proteins. Extracellular vesicles exert various effects on the maintenance of normal physiology including tissue repair, stem cell maintenance and blood coagulation. Exosomes are known to play a role in cancer, both in progression and as a prognostic value, found elevated in multiple cardiovascular diseases, diabetes mellitus. Exosomes are now being used in dentistry for tissue repair with Mesenchymal Stem cells regeneration, oral cancer detection and as saliva biomarkers for various diseases.

**CONCLUSIONS:** The apparent role of EVs in a vast number of biological processes, along with many of their intriguing features, forms the basis of extending EV analysis beyond basic research and into a clinical and therapeutic context. Despite being a relatively new field, the potential and versatility of Extracellular analysis are supported by an increasing number of publications. The applications of this type of analysis include the areas of diagnostics and prognostics, as well as drug therapy, regenerative medicine, and vaccines. For the purpose of diagnosis and prognosis, the use of EVs seems particularly promising because these vesicles contain a plethora of clinically relevant molecules, such as proteins and RNA from the parent cell. Thereby, the miRNA and protein patterns, which are unique for a specific pathologic condition, can be used.

## Dental Caries and Taste Receptor Genes

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**OBJECTIVES:** Dental caries is one of the most prevalent diseases worldwide and the most common chronic childhood disease in the United States. Although previous studies suggest the importance of genetic factors, not too much is known about dental caries in relation to food choices that people make due to a specific genetic mutation. Our review analyzes the genetic influence of taste perception on caries susceptibility. Specifically, we focused on the bitter taste perception from TAS2R38 genes and the sweet taste perception from the TAS1R2 genes. The results of this review proved that genotypes of taste perception do have an influence on dental caries. This information can be used to create a customized diet to prevent or decrease morbidity of dental caries in primary, mixed and permanent dentition.

**METHODS:** The twin study that examined the genetic component of caries looked at 44 pairs of twins and three triplets over a six-year period. The study used clinical and radiographic exams, study models and dental questionnaires. Another study looked at 46 pairs of monozygotic twins and 22 pairs of dizygotic twins reared apart to analyze teeth present, teeth present excluding third molars, teeth restored, teeth restored index, surfaces restored, surfaces restored index, and surfaces restored or carious. Other studies primarily used DMFT and DMFS scores to compare to the presence of TAS2R38, TAS1R2, and GNAT3 taste genes. For the study in Udaipur, India, data and blood or saliva specimens were collected from 54 patients with non-syndromic cleft lip and/or palate (NCLP), 57 relatives of patients with NCLP, and 38 unaffected controls. The Taqman allelic discrimination assay utilizing RT-PCR was used for rapid detection of five single nucleotide polymorphisms (SNPs) of TAS2R38 and TAS1R2.

**RESULTS:** Both twin studies showed a statistically significant correlation for monozygotic, but not dizygotic, sets of twins for percentage of teeth and surfaces restored ( $p < 0.001$ ) and for percentage of teeth and surfaces restored. Polymorphisms of taste genes associated with caries and NCLP in Udaipur, India, showed that the G allele can be considered as a protective allele for TAS2R38 taste receptor gene. Patients with NCLP in this study possessed less caries protective genes and more caries risk genes. DMFT and DMFS scores compared to presence of TAS2R38, TAS1R2, and GNAT3 study showed that certain alleles (G, G, and C) of the TAS2R38 gene provided protection from caries in the primary teeth alone.

**CONCLUSIONS:** The TAS2R38 genotype and bitter taste perception have a strong relationship to caries incidence. This knowledge can be used to target patients who are at a higher genetic risk for caries. The high-risk patients can be helped by a hygiene and diet program for caries prevention.

## **Mutations in Exons 1 and 2 of PAX9 Gene and Hypodontia**

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**INTRODUCTION:** Hypodontia is one of the most common dental anomalies that result in aesthetic and functional problems. A wide range of prevalence values for missing teeth has been reported ranging from 1.6% to 25.4% in adult population. Our understanding of the genetic basis of tooth agenesis is still limited. Several genes have been explored, including, but not limited to PAX9 and MSX1. The normal function of the PAX9 gene, a transcription factor that plays an important role in signaling between epithelial and mesenchymal cells during tooth development, seems to be critical during development of dental lamina.

**OBJECTIVES:** The goal of our study was to identify genetic mutations in exon 1 and exon 2 of PAX9 gene, commonly associated with a lack of tooth development in probands and their close relatives.

**MATERIAL AND METHODS:** Our sample consisted of 66 individuals with congenitally missing teeth and 50 of their relatives. Saliva specimens were collected from all individuals. Most of saliva specimens were collected using our own protocol. Modified chelex method was used to extract DNA. A smaller number of specimens were collected using Oragene saliva kit. Following DNA isolation, PCR was done using specific primers for each single nucleotide polymorphism, agarose electrophoresis followed to confirm PCR product, which was then purified and sent to sequencing laboratory. The sequenced specimens were analyzed for PAX9 genotypes.

**RESULTS:** Out of 66 individuals with missing teeth and 50 their family members that were genotyped, 13 mutations (9 in probands and 4 in family members) in exons one or two of PAX9 gene were found. Among 76 controls, none had a mutation in exons one or two of PAX9 gene.

**CONCLUSION:** Results of this pilot study suggest a rather strong association of exon 1 and exon 2 mutations of PAX9 gene in individuals with hypodontia in our sample. Evaluation of a larger sample will enable us to draw more definitive conclusions. Our study continues and, in addition to PAX9, also MSX1 and BMP4 gene polymorphisms are studied.

## **Cleft lip and palate in Vietnam and folate-related genes**

Holly Yuen<sup>1</sup>, Nick Ng<sup>1</sup>, Miroslav Tolar<sup>2</sup>, Veronika Tolarova<sup>3</sup>, Helen Berdichevsky<sup>1</sup>, Soomin Hong<sup>4</sup>, Fateh Arslan<sup>4</sup>, Melanie Seto<sup>4</sup>, Arief Halim<sup>4</sup>, Maureen Valley<sup>5</sup>, and Marie Tolarova<sup>4</sup>

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**OBJECTIVES:** The etiology of nonsyndromic cleft lip with or without cleft palate (NCL/P) is multifactorial. Folate-related genes, methylenetetrahydrofolate reductase (MTHFR) and reduced folate carrier 1 (RFC1), are among those genetic factors most intensively studied. When their function is altered due to mutations, a decreased utilization of folate slows down cell multiplication and it may contribute to orofacial clefting. RFC1 gene encodes a cell membrane protein essential for internalization of folate bound to a folate-binding protein. MTHFR gene encodes an enzyme that catalyzes formation of an active form of the internalized folate in the cell. The purpose of our study was to determine whether MTHFR677CT and the RFC180AG polymorphisms are associated with NCLP in CanTho, Vietnam.

**MATERIAL AND METHODS:** A case-control study design was used. Cases (individuals affected with NCL/P; n=38) and controls (n=33) for this study were identified during Rotaplast medical mission to CanTho, Vietnam. Diagnosis of NCL/P was determined by medical geneticist (MMT) conducting physical examination of each individual. Controls (n = 33) were recruited in the same hospital. Venous blood and saliva was obtained for DNA analysis. DNA was isolated from dry blood or saliva spots. MTHFR 677CT and RFC1 80AG genotypes were established by PCR amplification and single nucleotide conformational polymorphism detection using polyacrylamide gel electrophoresis (PAGE).

**RESULTS:** Mutations of the MTHFR 677th nucleotide were relatively rare in CanTho samples of cases and controls that we studied. We found a different proportion of genotypes in cases and controls for MTHFR 677CT, but only one homozygote TT (in controls). There were twice as many CT heterozygotes in cases compared to controls (34.2% vs 15.2%) and thus a higher T allele frequency in cases (0.171) compared to controls (0.106) was found. However, these differences were not significant. Very interesting findings were revealed by analysis of RFC1 80AG. Mutated allele G was common in cases as well as in controls (higher in cases - 0.554 compared to controls - 0.5). Although GG genotypes were observed in the same proportions in cases and in controls (27 % vs 27.3%), lower proportion of AA homozygotes (16.2% vs 27.3%) and higher proportion of AG heterozygotes (56.8% vs 45.4%) were observed. The difference in distribution of genotypes between cases and controls was statistically significant (p=0.041).

**CONCLUSION:** Our results suggest association of 80AT variant of the RFC1 gene with NCL/P. No association was observed for 677CT variant of MTHFR. The study continues by increasing size of samples for cases and controls and by involving also mothers and fathers of cases.

*Rotaplast International, Inc., funded and supported field work for this study. Pacific Craniofacial Genetics Team and Cleft Prevention Program and Department of Orthodontics funded and supported molecular genetics analysis.*

## **Non-invasive *in vitro* reduction of cancer cells through photodynamic therapy**

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**OBJECTIVES:** This study aims to provide a safe and more effective alternative for current treatment of head and neck cancers using liposomes and photodynamic therapy (PDT) on HeLa cervical carcinoma and HSC-3 oral squamous carcinoma cells.

**EXPERIMENTAL METHODS:** Liposomes were composed of palmitoylcholine (POPC):phosphatidylglycerol (PG), and contained either zinc phthalocyanine (ZnPc) or aluminum phthalocyanine chloride (AlPc). Free or liposome-encapsulated ZnPc and AlPc (0.1-1  $\mu$ M) were incubated with HeLa and HSC-3 carcinoma cell lines for 24 h at 37°C. The cells were then exposed to light (690 nm) from a High Power LED Multi Chip Emitter for 20 minutes. Cytotoxicity was analyzed by the Alamar Blue assay.

**RESULTS:** Analysis of the treatment on HSC-3 cells yielded a clear reduction in cell viability. Results indicate that the liposomal encapsulated form of ZnPc and AlPc were more effective than their free counterparts. At 1  $\mu$ M of free ZnPc and AlPc, cell viability was reduced to 75 $\pm$ 6% and 51 $\pm$ 1%, respectively. However, at 0.1, 0.5 and 1  $\mu$ M of encapsulated AlPc, cell viability was reduced to 54 $\pm$ 3, 20 $\pm$ 3 and 21 $\pm$ 2%, respectively. Similarly, at 0.1, 0.5 and 1  $\mu$ M of encapsulated ZnPc, cell viability was reduced to 89 $\pm$ 4, 69 $\pm$ 4, and 41 $\pm$ 5%, respectively. HeLa cells were similarly affected by the photosensitizers. At 1  $\mu$ M of free ZnPc and AlPc, cell viability was reduced to 53 $\pm$ 2% and 23 $\pm$ 5%, respectively. With 0.1, 0.5, and 1  $\mu$ M liposomal photosensitizers, the viability was reduced to 68 $\pm$ 9, 15 $\pm$ 10 and 0% (ZnPc), and to 26 $\pm$ 8, 0 and 0% (AlPc), respectively. The photosensitizers did not show dark cytotoxicity in either cell line.

**CONCLUSION:** Based on the results of this study, PDT in tandem with liposomal delivery may prove to be a safer treatment modality for head and neck cancers. The liposomal forms of ZnPc and AlPc effectively reduced cell metabolic activity and even more so than their free counterparts. Future experiments will focus on increasing effectiveness of the photosensitizers through encapsulation in folate-conjugated liposomes.



# **IDS STUDENT REVIEW PRESENTATIONS**

## **Traditional vs digital impressions in fixed and implant dentistry**

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**OBJECTIVES:** Impression taking is one of the most important procedural steps in fabrication of fixed prosthetics and implants. Dental profession has been employing traditional techniques to acquire impressions but currently digital impression capturing systems are also increasingly being available. We compared the traditional and digital methods for acquiring impressions in fixed and implant dentistry. We looked at the advantages and disadvantages of both methods, studied the current digital systems available in the market and synthesized the current evidence for their use in implant dentistry and fixed dentistry.

**METHODS:** Studies comparing the accuracy, patient comfort, time, cost, learning curve, storage and cleanliness were included. A comparison of major digital impression systems available in the market and their features was done. Step by step assessment of the digital work flow involved in digital impressions for implants was conducted. Studies comparing the two procedural techniques when used in fabrication of crowns and bridges in fixed dentistry were collected. Evidence was synthesized for accuracy, marginal fit, internal fit, clinical chair side evaluation and survival of crowns. Errors encountered at different steps in the fabrication of crowns and bridges for both techniques were highlighted.

**RESULTS:** Traditional impression taking techniques were found to be messy and 36% of dentists reported retaking the impressions 3 or more times in a month. Some studies indicated that digital systems were less accurate than traditional impressions. In terms of patient preference, 100% patients preferred digital impressions due to comfort. The functions of currently available digital systems CEREC, iTero, E4D, Lava COS, True Definition Scanner, Trios, IOS fast scan were highlighted.

**CONCLUSIONS:** Traditional digital systems are the tried and tested system and have been in use for many years. Digital impressions were favored by patients for comfort and ease of procedure. They are less messy. Cost was found to be a major factor in adoption of techniques. Open file sharing systems are leading to an increase in the adoption of digital impression technology. Case selection is a driving factor in choosing one technique of impression taking over another. It is up to the personal preference of a practitioner in ultimately deciding to use one technique or the other.

## **Caries process and preventive strategies**

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**OBJECTIVES:** The caries disease process currently is a multifactorial disease which if targeted through different preventive and treatment mechanisms. A current literature review of the caries process will guide the reader throughout its evolution and current understanding of the disease. Its relevance lies on the fact that there is a need to create different preventive strategies which target the cause versus the effect. The new ADA caries classification allows for diagnostic methods which will allow us to further utilize CAMBRA products to reduce the level of risk in our patient population.

**METHODS:** Literature review of more than 50 different articles using PubMed and/or scientific journals as our primary resources. Keywords: caries, early prevention of the caries disease, classification today, etiology of caries disease process, CAMBRA, early diagnosis of caries. Inclusion criteria: not older than 2009, RCT, systematic reviews, scientific relevance. Exclusion criteria: older than 2009, clinical cases, series of clinical cases. We mainly focused on the new classifications, terms, preventions and products available to prevent the caries disease process.

**RESULTS:** An uncountable number of bacteria is involved in the caries disease process. Not only *Streptococci Mutans* and *Lactobacilli* but hundreds more. The etiology and risk factors vary for each individual hence the need to treat each patient accordingly. Most articles pointed out that the earlier the diagnosis and prevention the better, and with this statement it was determined that there are many new ways to detect and diagnose caries. Finally, there are many CAMBRA products; each one with different purpose and indications for each patient.

**CONCLUSIONS:** Dental caries is a multifactorial disease, which makes it difficult to treat. SM and LB were once considered the principal microorganisms causing the caries disease, but after this review we can conclude that many other organisms also play an important role in the development of the disease. New diagnostic methods, which contribute to the accurate detection of the dental caries, are being continually introduced to the market. Caries is a preventable disease and CAMBRA is so far the most organized protocol to help eliminate it, even though there is a need for a shorter and simpler system to use in private practice. Fluoride continues to be the leading product to prevent caries disease. Xylitol is also an excellent choice as well. More research is needed to find new products, which have a broader spectrum of action on the bacterial cell specifically.

## Teeth whitening

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**OBJECTIVES:** There have been significant technological advances in the field of dental bleaching over the last 10 years that have made a corresponding increase in the number of materials available. Improvements in clinical performance longevity and ease of application have made bleaching products more popular and more predictable. Despite improvements. However as with any dental procedures bleaching involves risks too. The authors of this poster aim to discuss the different types of bleaching procedures for vital as well as non-vital teeth. The poster would also include side effects of bleaching procedures on soft and hard tissue. In this review, we conducted a comprehensive literature review to compile and compare different types of bleaching procedures.

**METHODS:** A comprehensive review of the literature was completed seeking evidence for the treatment of teeth with all-ceramic restorations. A search of peer-reviewed literature was undertaken using PubMed, MED-LINE, JADA and CDA journals with a focus on evidence-based research articles published between 1974 and 2015. A hand search of relevant dental journals was also completed. Randomized controlled trials, nonrandomized controlled studies, longitudinal experimental clinical studies, longitudinal prospective studies, and longitudinal retrospective studies were reviewed. Data supporting the clinical application of all-ceramic materials and systems was sought.

**RESULTS:** The literature demonstrates that multiple materials and systems are currently available for clinical use. The successful results without any side effects depend on the identification of the stains and determination of the proper concentration of the agent so as to prevent any side effects. A close understanding of the components of the bleaching agents of different materials is necessary for obtaining desired results.

**CONCLUSION:** New generation bleaching products present dentists with many restorative options in these exciting times for dental cosmetics. Many companies promote their products and are leaning or advocating for unsupervised bleaching process. However we need to understand that their some deleterious effects too for bleaching. It becomes imperative on our part to educate our patients so that they don't get lured by such products. Companies want to be part of such emerging market. However, due to technological advancements lot of procedures have exhibited minimal side effects with maximum desired results. Definitely, future is bound to witness a bigger revolution in field of dental bleaching, with introduction of newer materials to keep up the pace with growing cosmetic needs.

## **Dental erosion and tooth wear**

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**OBJECTIVES:** Dental erosion has been traditionally thought of as tooth wear caused exclusively by chemical means, which distinguished it from attrition, abrasion and abfraction. It is a growing concern for dental practitioners and their patients as we are seeing a rise in its occurrence, especially among children and adolescents. The authors of this poster aim to discuss current clinical research that shows a synergistic relationship among dental erosion and other types of tooth wear. This poster will cover intrinsic and extrinsic factors that contribute to tooth wear. It demonstrates how behavioral factors such as nutrition, lifestyle choices, oral hygiene practices and certain occupations can contribute to tooth wear. Biological considerations such as the oral environment and systemic conditions will also be discussed. Additionally, this poster will present the different classifications of tooth wear, as well as the prevention and treatment modalities that are available for patients today.

**METHODS:** A comprehensive review of dental and medical literature was completed seeking evidence for current advancements in the classification and treatment of dental erosion and other types of tooth wear. A search of peer-reviewed literature was undertaken using PubMed, CDA journals, and international dental journals with a focus on evidence-based research articles published between 2011 and 2015. Randomized controlled trials, nonrandomized controlled studies, longitudinal experimental clinical studies, and longitudinal prospective studies were reviewed. Data supporting the advancements in dental erosion, its prevention and treatment were sought.

**RESULTS:** The literature demonstrates that unlike the traditional categories of tooth wear which segregated erosion, attrition, abrasion and abfraction into individual entities, tooth wear is a synergistic combination of these destructive forces. The resulting wear can be treated depending on the amount of tooth destruction. Current prospective clinical trials have been geared towards less invasive procedures, such as the veneered sandwich technique, to restore esthetics and conserve the remaining tooth structure. Emphasis was placed on the clinician's responsibility of early recognition of tooth wear, as it is crucial to determine the etiology and establish means to prevent further destruction.

**CONCLUSION:** Current research has discovered that dental erosion, combined with abrasion, attrition and abfraction, is a synergistic and multifactorial process. It is caused by both behavioral and biological factors. As dental practitioners, it is our responsibility to recognize the erosive process early on; educate our patients on changes they can make to prevent the progress of erosive wear; intercept and restore as necessary; and maintain the dentition of patients with erosive issues.

## **Endodontic Therapy vs Single Unit Implant**

Amin Samadian, Min Wei, Amir Sanjabi, Azita Kabiri, Nino Kobahidze.  
Faculty Advisor: Kenneth E. Moore

**OBJECTIVES:** The treatment of a single tooth can be accomplished using endodontic therapy or extraction and replacement with an implant restoration. Historically, endodontic therapy was considered the primary modality to save failing teeth by using primary therapy, retreatment, or surgical approaches. Once these endodontic options were exhausted, the tooth would be deemed unrestorable and be extracted. Today, with the advent of implants, modern clinicians have an alternative for ailing teeth. Sometimes, the prognosis of the tooth yields a clearly superior treatment; however, quite frequently the tooth falls into a grey area. Multiple factors must be considered and critical thought is required to give patients the best treatment. A review of the literature was done to determine some factors favoring each treatment in an equivocal situation.

**METHODS:** A comprehensive review of the literature was done to determine which factors influence treatment decisions in a single tooth scenario. The review was performed on 55 articles containing, randomized control trials, non-randomized controlled studies, longitudinal prospective, and retrospective clinical studies. The search of these peer-reviewed journals was achieved using PubMed, MED-LINE, JADA and CDA journals with a focus of evidence-based research articles published between 1995 and 2015. Of the 55 articles selected based on the above criteria, there were 10 systematic review articles, which reviewed approximately 150 articles each.

**RESULTS:** Of the influencing factors four groups emerged as focused by the literature. These were found to be over-all survival and success rates, systemic factors, local factors, and patient specific factors. The direct comparison between endodontic therapy and implant replacement of single unit teeth was found to be challenging due to the vastly different and diverse definitions of success and survival throughout various studies. It appears that the survival rate of single unit implants and endodontic therapy are both over 95%. The success rates has a range 80% to 96% depending on the study. Poor systemic health tends to adversely affect both treatments; however, endodontic therapy appears to be slightly favored in the medically complex individual. Local factors such as poor periodontal health, poor remaining tooth structure, or high prosthetic demand tend to favor implant therapy. Additionally, while initial endodontic therapy and single tooth implant replacement appear to be comparable, retreatment and surgical treatment has significantly lower prognosis. Finally, patient specific factors including quality of life, esthetic, and patient age appear to be equivocal between treatments; however, endodontic therapy has less financial cost and reduced treatment time compared to implant therapy.

**DISCUSSION:** When evaluated globally on multiple levels, both treatment modalities have similar and favorable outcomes. The answer of which is the superior treatment was difficult to ascertain as the literature has multiple biases and confounders, as well as non-standardized definitions. The largest differences occurred when the restorability of the tooth was assessed appropriately and the individual needs of the patient were considered. Therefore, to make an accurate recommendation between the treatments, an accurate diagnosis and prognosis of the tooth in question must be completed. This will place the tooth on a spectrum with implant

replacement and endodontic therapy on either end. When the scenario is located near the extremes, the dentist should advocate the clearly indicated treatment. When the scenario lands in the middle of the spectrum and evidence based arguments can be made for either treatment, the dentist should educate the patient of the risks, benefits, and alternatives. It should be the patient armed with the consult of the dentist making the decision.

**CONCLUSION:** The treatment decision between endodontic therapy and implant therapy is complex and multifactorial. As both treatments have very positive outcomes, an accurate diagnosis and prognosis of the tooth is critical when selecting the treatment for a patient's best care.

# **STOCKTON CAMPUS STUDENT PRESENTATIONS**



## **Transmissibility of oral bacteria: are we sharing too much?**

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**OBJECTIVES:** The purpose of this study was to determine if oral bacteria adhere to a used tube of toothpaste after four days of use compared to an unused tube of toothpaste. Our hypothesis was that if oral bacteria adhere to the used tube of toothpaste, then it is possible that the bacteria can be transmitted onto any toothbrush sharing the same tube of toothpaste. Our null hypothesis is that oral bacteria do not adhere to a used tube of toothpaste after four days of use, and that sharing toothpaste does not promote transmission of bacteria.

**METHODS:** This study was conducted in three phases. Phase I: Forty-one students, faculty and staff from the Pacific Dental Care Clinic at the University of the Pacific in Stockton California were asked to have their lower anterior teeth swabbed for their bacterial count level using the CariScreen meter. Phase II: Participants were given a bag of items containing: one manual soft bristle toothbrush, two tubes of toothpaste (one to be used (test) and one not to be used (control)), and one brushing data sheet. Participants were asked to brush their teeth morning and night for four days. Half of the participants were asked to keep their items in the bag provided when not in use and the other half of the participants were asked to keep their items outside of the bag when not in use. Phase III: Twenty-nine participants followed through with phase II of this experiment. All test and control tubes of toothpaste were inoculated, plated and incubated using a Lysogeny broth with agar in a laboratory setting to determine bacterial growth.

**RESULTS:** We found that there is a correlation between oral bacteria having the ability to adhere to a used tube of toothpaste ( $m = 1.48$ ,  $s = 4.718$ ) compared to an unused tube of toothpaste after four days of use ( $m = 0.31$ ,  $s = 0.660$ ),  $t(28) = -1.331$ ,  $p > 0.05$ . We reject our hypothesis and retain the null hypothesis that oral bacteria do not adhere to a used tube of toothpaste compared to an unused tube of toothpaste after four days of use. Our results indicate that sharing tubes of toothpaste does not contribute to the transmission of oral bacteria.

**CONCLUSION:** Further research needs to be conducted using a larger sample size and should include a more representative sample of the general population. It would be beneficial to sample the tubes of toothpaste at different time intervals after use. In order to see when the bacteria is thriving the most and at what time interval the bacteria can no longer survive outside of the mouth. Potentially determining at what time interval it is least likely to transmit bacteria when opting to share a tube of toothpaste. Laboratory protocols need to be improved and standardized.

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## The structural characterization of the *Saccharomyces cerevisiae* $\alpha$ mat $\alpha$ secretion signal

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**OBJECTIVES:** The MAT $\alpha$ , a secretion signal leader, is an 85 amino acid region helps provide a high level of secretion when it is fused to the N-terminus of most proteins. However, limitations exist as some proteins cannot be secreted efficiently even with the MAT $\alpha$  prepro secretion signal. This study focuses on using deletion mutagenesis of specific sets of amino acids of MAT $\alpha$  to evaluate the correlation between secondary structure and secretion level. By creating mutant MAT $\alpha$  prepro signal leaders and analyzing their physical properties, we hope to better understand the signal leader's structural properties in order to further increase secretion levels of heterologous proteins in *P. pastoris*.

**METHODS:** To physically analyze the actual secondary structure of the MAT $\alpha$   $\Delta$ 57-60, the mutant MAT $\alpha$  was initially cloned by PCR, ligated into a pET SUMO vector, and expressed in BL21 *E. coli*. After small-scale expression to optimize conditions for large-scale expression, the MAT $\alpha$   $\Delta$ 57-60 was purified by affinity chromatography, dialysis, and SUMO digestion. The purified mutant MAT $\alpha$ 's secondary structure was then determined by circular dichroism and compared to that of wild type MAT $\alpha$  peptide.

**RESULTS:** The mutant MAT $\alpha$   $\Delta$ 57-60 peptide was successfully expressed and purified. Circular dichroism results show a significant decrease in  $\beta$ -strand amount compared to the wild type peptide.

**CONCLUSIONS:** The results are consistent with the predicted model that shows existing beta strands associated in amino acids 50-70. It can be strongly inferred that the deletion of the  $\beta$ -strand (57-60) caused significant change in the mutant MAT $\alpha$  secondary structure and caused the decrease in the protein secretion level. The understanding and possible improvement of the MAT prepro signal peptide through modification of the pro-peptide have great potential in optimizing the secretion systems of microbial host organisms, like *Pichia pastoris*. The optimization of secretion systems in recombinant hosts will impact fields of science, industry, healthcare and economics worldwide.

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## **Apitherapy for oral conditions: The use of bee products to improve oral health**

Katherine Stratton

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**OBJECTIVES:** Apitherapy, and other complementary and alternative medicines (CAM), are of growing interest amongst health care consumers. There is little familiarity in this area in regards to the safety and effectiveness of the proposed treatments. This paper aims to raise awareness of apitherapy and address the interest of dental professionals to understand the potential benefits and risks of CAM.

**METHODS:** Review of clinical research and evidence revealing that some bee products are a cost effective and natural alternative to modern medicine that can be used in dentistry and a survey of dental students and professionals at the Arthur A. Dugoni School of Dentistry to evaluate awareness and perspective toward research and education.

**RESULTS:** Clinical evidence was found showing that certain bee products are effective at improving oral health and can be developed into products for consumers. Students and faculty at the Arthur A. Dugoni School of Dentistry overall were not familiar with the use of bee products in dentistry, and felt that more research and education on CAM was important in health care.

**CONCLUSIONS:** Certain bee products are a cost effective and natural alternative to modern medicine that can be used in dentistry. CAM is an area of medicine where more research is needed in order to inform and benefit our patients.

## **E-cigarettes: if you can't pick a side, pick knowledge**

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**OBJECTIVES:** E-cigarettes are fast growing phenomenon in today's society. Although the ADA, FDA and other influential health organizations have not sided or chosen a position in regards of e-cigarettes it is important that dental professionals possess the knowledge to better inform patients in regards of this topic. Dental professionals are required and expected to advocate for oral and systemic health but yet e-cigarettes are not required in the curriculum for dental, dental hygiene or dental assistants. It is not necessary that the dental professionals chooses a side but it is crucial that the dental professionals becomes familiar with e-cigarettes since it can potentially become a weapon against several diseases or detriment the health of society by introducing a new harmful device.

**METHODS:** A selection of academic journals and various informational sources were used to find information that could educate dental professionals. The research was used to validate that there are many facts and myths that many dental professionals are not aware of and that could potentially benefit a patient who is an e-cigarette user, a patient interested in tobacco cessation or a patient who is interested in starting to use the e-cigarette device. A small survey of 50 dental professionals including dentists, dental hygienist and dental assistants was also conducted asking questions in regards of their knowledge and opinion on e-cigarettes.

**RESULTS:** The research validated that although there is further research to be made in order to determine the beneficial or harmful properties of e-cigarettes, there is a mass of information and facts that need to reach the public. Dental professionals are advocates of oral and systemic health as well as tobacco cessation counseling, which makes us ideal ambassadors of providing our communities, and patients with knowledge on e-cigarettes and their consumption. The survey disclosed that 80% of the subjects were not aware of the ADA stand on e-cigarettes, 10% believed the ADA does not support e-cigarettes and only 10% were correct in answering that the ADA does not currently have a stand on cigarettes. Other questions validated the little thought dental professionals have given to e-cigarettes and the fact that they are becoming part of a new generation.

**CONCLUSIONS:** The e-cigarette is a topic that must be added to the curriculum of the dental professionals. While further research is being done in order to determine the properties of cigarettes, dental professionals must be educated about the known properties of the e-cigarette so that the public can be better informed and be able to make an informed decision about the consumption of e-cigarettes. The education about e-cigarettes must be provided from continuing education courses, lectures, pamphlets, articles, and other events such as Research Day at the University of the Pacific.