

ABSTRACTS

I. PREDOCTORAL CATEGORY

#01 Title: In-vitro study of a cationic cement and its effect on dentin remineralization

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Objectives: Polyallylamine hydrochloride (PAH) is a cationic polymer that interacts with calcium ions and has shown potential to remineralize collagen fibrils and dentin matrices.

The aim of the project is to develop a novel glass ionomer cement comprised of PAH for application towards dentin remineralization. It will be achieved by the following objectives:

1. Test different formulations of cement components to facilitate cement setting.
2. Optimize composition to allow for setting times to be clinically acceptable.
3. Test the effectiveness of selected formulation of the cationic cement in remineralizing artificial lesions in dentin of extracted teeth in vitro.

Methods: Different combinations of calcium and phosphate source such as Bioglass, calcium phosphate dihydrate, polyallylamine hydrochloride (PAH) and deionized water will be tried to get a desirable consistency of the cement. Extracted teeth will be used as samples which will be cut into round discs of dentin, removing the enamel portion. The discs will then be coated with the nail paint leaving a window of 2x2mm to prevent demineralization of the undesired surface and put in demin solution for 66 hours to create artificial demineralized lesions. The cement will be placed on the demineralized surface and after setting of the cement the samples would be immersed in the remin solution for 14 days. The samples will then be assessed under high power electron microscopes where evidence of remineralization will be detected.

Results: Cement with clinically acceptable consistency and setting time was formulated. PAH 30mg, Deionized water 49l, Calcium phosphate 60mg and Bioglass 60mg was used for the cement which took about 11mins to set. The remineralization of the demin discs with cement and remin solution was significantly higher when compared to the remineralization of the discs with remin solution only.

Conclusion: PAH can be used to make a glass-ionomer cement that sets at reasonable times.

Support: UCSF Catalyst Award "PILP Treatment for the Repair of Dental Caries" and by the Center for Dental, Oral & Craniofacial Tissue & Organ Regeneration (C-DOCTOR) with the support of NIH NIDCR (U24DE026914)

#02 Title: The effect of different RUNX2 isoforms on cellular proliferation

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Objective: The relatively long-billed white Pekin duck (*Anas platyrhynchos*) and relatively short-beaked Japanese quail (*Coturnix japonica*) use the same conserved molecular and cellular mechanisms to create clear differences in skull and beak morphology. Understanding how these two species modulate the function of genes that regulate jaw development is critical to understanding how the same genes might produce jaw defects in humans. One gene known to be expressed by neural crest cells, which form the jaw skeleton is Runx2. Runx2 has been shown to have several functions including promoting cellular proliferation and driving osteogenesis. There are at least eight different Runx2 isoforms in duck generated through alternative splicing. How these different isoforms differ in function and regulation is poorly understood. These isoforms contain different domains including variation in Q/A repeats, VWRPY motifs, and an NMTS domain that may affect their function. We seek to determine what effect different Runx2 isoforms have on cellular proliferation.

Methods: Chick fibroblast cells (DF-1) were transfected with 8 Runx2 isoform overexpression constructs along with an empty vector control. A colorimetric MTS assay was used to measure cellular metabolism over a 3-day time course. A T-Test was used to identify significant differences between empty vector control cells and isoform overexpression cells.

Results: Duck isoform "D123QF" resulted in the highest difference in proliferation when compared to the empty vector control ($P < 0.0001$). "D123QF" had an absorbance fold change value of 2.73 while the empty vector control had a value of 1.78. All untagged duck isoforms tested showed a significant difference in proliferation when compared to the empty vector control ($p < 0.05$).

Conclusion: Runx2 drives cellular proliferation but not all Runx2 isoforms are equal in directing proliferation. In this experiment, we have indicated that there is a link between genetic variation due to splicing and the rate at which cells proliferate.

Support: The Associate Dean for Research Student Research Fund and NIH Grants R01DE025668 and R01DE016402.

#03 Title: Third party reimbursement manipulation among dental professionals in California

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Objectives: This cross-sectional study surveyed CDA affiliated dentists to examine the difference between the amount of dental insurance coverage patients have and the dental care they need, because dental insurance is offered by corporate insurance agencies that are detached from everyday workings of dental practices.

Methods: 4,254 CDA affiliated, dentists were randomly selected to participate. Our instrument was developed in Qualtrics and contained multiple choice and case-based scenarios. In each hypothetical situation, participants indicated their willingness to make intentional billing errors to allow the patient to better afford care. Answers were reported on a five-point scale. Chi-squared tests were performed to identify relationships between responses to patient scenarios and demographic information such as, practice setting, age, and dental specialty.

Results: 414 responses were collected yielding a 9.7% response rate. 74.4%, $\chi^2(6, n = 414) = 48.07, p < 0.05$, of respondents reported that they always or very often find that policies from dental insurers hinder the level of care that they would like to provide (General: 77.7%, OS: 77.8%, Endo: 33.3%, Peds: 81.3%, Ortho: 25%, Perio: 73.3%, Prosth: 75%). 31.4% reported that they were likely or extremely likely to adjust insurance billing and coding to provide patients with necessary care that they could not afford otherwise.

Conclusion: The degree at which participants felt limited by insurance policies was most effected by their specialty. This is likely due to the quantity of elective procedures different specialists perform. Age and practice setting did not affect responses with statistical significance suggesting that this issue is deeply ingrained in the current dentistry model. Future steps are necessary to define an appropriate blueprint for policy development that addresses the needs of practicing dentists and their patients.

Support: Lee Hysan Fund, Dr. Alfa-Ibrihim Yansane

#04 Title: Charge-modified 14P2 peptide of amelogenin assembles nanoribbons

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Objectives: Amelogenin is a critical protein in the developing enamel matrix, and its 14P2 region is significant in its ability to adapt beta-sheet configurations and to self-assemble into amyloid-like ribbons. This project aims to evaluate the self-assembly kinetics of a charge-modified variant of the 14P2 peptide and tests if an extension of the peptide with negatively charged residues will facilitate guided mineralization along peptide ribbons.

Methods: The amelogenin-derived 14P2 peptide was modified with four glutamic acid (P2-4E) residues at its C-terminus. P2-4E and its phosphorylated variant, pP2-4E, were incubated in ionic calcium phosphate solutions at pH 4.5 - 5.5 and samples were prepared and imaged by atomic force microscopy (AFM) on mica glass. Samples with nanoribbons were treated with ZnO and ZnS solutions and analyzed by atomic force microscopy (AFM).

Results: P2-4E self-assembles into nanoribbons under calcium phosphate conditions. The nanoribbons exhibit variations in length ranging from the 100nm range up to micrometer scale lengths in addition to bundling and twisting morphologies. The addition of ZnO and ZnS mineral did not yield signs of patterned attachment to the nanoribbons. By contrast, its phosphorylated variant forms primarily amorphous aggregates and occasionally shorter rod-like structures under calcium phosphate conditions, with ribbons at the micrometer scale being considerably rare. In the absence of phosphate ions however, only amorphous aggregates were observed.

Conclusions: The addition of charged glutamic acid residues does not inhibit the nanoribbon self-assembly process. The modifications, however, are insufficient for mineral nucleation studies on a mica surface. Adding glutamic acid residues also affects the phosphorylated peptide's self-assembly, with complete inhibition when phosphate ions are removed from the medium.

Support: UCSF School of Dentistry Summer Dental Student Fellowship Program; NIH-RO1DE025709.

#05 Title: The economic impact of tobacco use on oral healthcare costs

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Objectives: Development of periodontitis and oral cancer can be attributed to tobacco use. However, the associations between tobacco use and oral healthcare costs have been poorly examined. We aim to assess the associations between different tobacco use patterns and dental care expenditures (DCE).

Methods: We obtained and linked National Health Interview Survey-Medical Expenditure Panel Survey (1998-2010) and identified each subject as a never/current/former and single/mixed user. Current/former vs. never-cigarette smokers were subclassified with similar propensity scores into 6 subclasses. Due to a small sample size, we established matched sets for cigar and smokeless current/former/never users and cigarette single/mixed users. DCE was compared with Wilcoxon rank sum test within each subclass/matched set. Overall DCE was estimated as the weighted average difference in median DCE between smokers and never-smokers (weight=N/subclass).

Results: For current/never-smokers, from subclasses 1-6, we observed decreasing proportions of Hispanic, ≥65-years, college graduate, high family income, privately insured, lifetime abstainers, having flu shot, and seatbelt use. Within subclasses 2-5, never-smokers had small higher DCE than current smokers (p-values between <0.0001 and 0.046). Overall weighted average difference in median DCE between current and never-smokers was \$0.

For former/never-smokers, there was no significant DCE difference within subclasses 1-4, while within subclasses 5-6, never-smokers had small higher DCE than former smokers. Overall weighted average in median DCE for former smokers was \$7.62 higher than never-smokers.

DCE did not differ significantly for current/former cigar and former smokeless tobacco users, compared to never-users (p=0.929, 0.267, 0.129), while current smokeless tobacco users spent less DCE than never-users (p=0.007).

No significant difference in DCE was found between current/former cigarette single and mixed users (p=0.452, 0.576).

Conclusion: Smokers in some subclasses seemed to pay less attention to oral health than never-smokers. Promotion of awareness of oral health among smokers is crucial to improve their long-term oral health.

Support: Program in Global Oral Health

#06 Title: Near-infrared image guided laser ablation of root surface lesions

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Objective: Near-Infrared (NIR) reflectance at wavelengths above 1500-nm can be used to distinguish between sound and demineralized tooth structure. Cross polarized optical coherence tomography (CP-OCT) can be used to measure the depth of such lesions. Previous studies have shown that these imaging modalities, combined with a CO₂-laser, can effectively remove coronal lesions, but its use in removing root surface lesions needs to be further investigated. This study evaluates whether simultaneously scanned NIR and CO₂-lasers can be used to selectively remove root surface lesions.

Methods: Samples with natural root surface lesions were selected by verifying demineralization via NIR reflectance at 1500-1700-nm, and digital microscopy. Next, CP-OCT images were taken across sample lesions. Samples were then scanned using a NIR fiber laser to identify the region of demineralization. This image was used to selectively guide the CO₂-laser in removal of the lesion. Two groups were investigated, one with and one without dynamic focusing, i.e. changing the z-axis position of the tooth prior to imaging between ablation. Initial and final CP-OCT images were generated to analyze change in lesion depth. Initial and final NIR images were analyzed to assess the area of selective lesion removal.

Results: CP-OCT indicated that the laser removed 53% of the depth of the root surface lesions (n=9) without dynamic focusing, while it removed a significantly higher percentage, 86%, of the depth of root lesions (n=8) with dynamic focusing. CP-OCT images displayed a clear reduction in lesion depth, while NIR reflectance images corroborated the removal of demineralized areas on root surfaces.

Conclusion: NIR imaging modalities have proved capable of obtaining high contrast images of demineralized root surfaces. The findings indicate that the use of an image guided approach is highly capable of selectively removing lesions from root surfaces.

Support: Associate Dean for Student Research Fund and NIH/NIDCR grant RO1-DE19631

#07 Title: Understanding axon patterning in secondary palate development

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Objectives: Cleft secondary palate can lead to difficulties in effectively anesthetizing patients presumably due to axon patterning variations. Efnb1, mutations in which cause craniofrontonasal syndrome, binds to tyrosine kinase EPHB receptors in a bidirectional signaling system which can induce the collapse of axon growth cones. While Efnb1 is necessary for secondary palate development, its role in axon patterning in this context is unknown. The aim of this project was to understand axon pattern formation during palate development and determine how candidate regulator Efnb1 influences axon patterning.

Methods: Efnb1 Δ /Y, Efnb1+/-, and wild-type mouse embryos were generated and genotyped according to approved animal protocol. Immunostaining was conducted on whole mount palatal explants, cleared with BABB, and imaged using confocal microscopy to observe patterns of innervation.

Results: Immunostaining of wild-type palatal explants at E11.5 against neurofilament 2H3 showed that the greater palatine nerve (GPN) begins as a thick axon trunk with few branches. Ephrin-B1 is expressed in the anterior palatal shelves, correlating with the direction of GPN outgrowth. From E12.5 to E15.5, branching increases laterally, then medially. At E13.5, Efnb1 Δ /Y had decreased branching from the main trunk and appeared to have smaller palatal shelves (n=1) relative to wild-type littermates (n=3). Moreover, Efnb1+/- (n=3) exhibited smaller branches at E15.5 compared to control littermates (n=3).

Conclusions: Although our number of samples is insufficient to make a definitive conclusion, comparison of heterozygotes between E13.5 and E15.5 suggested that at E13.5 Ephrin-B1 expressing regions did not have axon branching while at E15.5 Ephrin-B1 expressing regions had increased branching. This suggests that Ephrin-B1 may play a repulsive role at E13.5 and regulates axon branching at E15.5. Further studies will be required to understand how these nerves are guided to their targets, and ultimately enable us to improve dental therapeutic management of patients with orofacial clefts.

Support: Department of Cell and Tissue Biology, R01DE025877

#08 Title: Antibiotic stewardship in dental implantology

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Objective: The Center for Disease Control recommends that outpatient facilities implement antibiotic stewardship activities and the American Dental Association (ADA) has adopted an evidence-based approach for antibiotic use. We aim to improve antibiotic stewardship in the UCSF Oral & Facial Surgery Center (OFSC) by analyzing prescribing habits to ensure appropriate antibiotic selection, dosage, frequency, and duration, and to limit overuse and eventual resistance and/or patient sensitization.

Methods: An Appointment Profile Report was generated from WINOMS OMFS Practice Software on all implant cases performed in the OFSC from 5/11/16 to 5/11/17. The Committee on Human Research—UCSF's Institutional Review Board—granted approval. A data collection form was developed and utilized during the review of the electronic medical records for patients identified in the report.

Results: Between 05/11/16 and 05/11/17, the OFSC performed 356 implant surgeries. Of 192 cases where pre-operative antibiotics were prescribed, 181 cases were Amoxicillin (averaging 956mg). Of 172 cases where post-operative antibiotics were prescribed, 168 cases were Amoxicillin (averaging 500mg, three times a day for 6.8 days). All remaining cases either did not involve antibiotics or did not have adequate documentation to assume otherwise. Using Misch and Resnik's Prophylactic Protocol guidelines for implants, 244 of the 356 cases were considered 'Category 1,' 17 'Category 2,' 27 'Category 3,' 4 'Category 4, and 0 'Category 5.'

Conclusions: Oral surgeons at the UCSF OFSC are prescribing dosages consistent with Misch and Resnik's recommendations for pre-operative antibiotics but are exceeding recommended amounts for post-operative antibiotics. This suggests that antibiotic stewardship can be improved. To establish guidelines for prescription of antibiotics following implant placement, the ADA could create a uniform accepted standard. However, it is the surgeon's prerogative to determine the appropriate course of antibiotics as every patient presents with their own circumstances that may increase their chances of developing an infection.

Support: Department of Oral and Maxillofacial Surgery

#09 Title: Bridging the gap between patient care x-ray-CT and micro x-ray-CT

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Objective: Patient care-X-ray computed tomography machines (XCT) are end-stage diagnostic units with large fields of view, low resolving capacities (230-300 μ m) and often cannot visualize beginnings of ectopic calcifications or dental lesions. Laboratory-benchtop micro X-ray CT (micro-XCT) machines have higher resolving capacity (<1 μ m), capable of investigating beginnings of pathologic occurrences at higher detail but only ex vivo; imaging following extraction of calcified mass from its context. This project aims to contextualize compositional heterogeneity within pathologic minerals by correlating quantitative data sets obtained from XCT and micro-XCT systems.

Methods: Structural (CT and mineral density (MD) maps of phantoms, human mandible, and aortic plaque using XCT (GE Discovery CT-HD) and micro-XCT (Zeiss Micro XCT-200) were obtained. Each cylinder of the two-part phantom with concentric cylinders (inner and outer) ranged in segments of known MD(0, 100, 250, 500 and 750 mg/cc) was used to calibrate both XCT and micro-XCT.

Results: Air and water XCT Hounsfield Units (HU) were -1000 and 0, and for micro-XCT were 0 and 1000. The HU values of the phantoms (in the increasing order of MD) scanned using XCT and micro-XCT were as follows. XCT-HUouter phantom were 1030, 1256, 1525, 2160 and 2542. XCT-HUinner phantom were 1012, 1220, 1767, 2419, 2694; micro-XCT-HUinner phantom were 715, 1238, 1902, 2967, 4368, 13,528. Subsequently, HU vs. MD calibration curves for XCT and micro-XCT illustrated MD of human specimens: XCT-mandible:615 \pm 451mg/cc, micro-XCT-tooth:1471 \pm 451mg/cc, XCT-aortic plaque813 \pm 400mg/cc, micro-XCT-aortic plaque: 1046 \pm 230mg/cc.

Conclusion: Results indicated XCT flux can delineate macroscale structures with lower MD. Flux of micro-XCT delineates microscale structures and their chemical composition. Correlation of data sets obtained from XCT and micro-XCT provides insights into mineralization foci at site-specific anatomic locations. This study supports developing plausible methodologies between XCT and micro-CT to contextualize biopsy material through which origin of pathologic processes in humans can be discovered.

Support: Department of Oral and Maxillofacial Surgery, School of Dentistry, School of Medicine, NIH/NIDCR R21DE027138 (SPH)

#10 Title: 3-D Analysis of Buccal Cortical Thickness in Individuals with UCLP

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Objectives: The CDC estimates that 7,088 babies are born each year with an orofacial cleft, making it the most common birth defect in the United States [1]. Due to the incomplete growth of the maxillary process, it is not uncommon for unilateral cleft lip and palate (UCLP) patients to present with maxillary hypoplasia leading to class III malocclusion. The goal of this study is to evaluate

buccal cortical plate (BCP) thickness in individuals with non-syndromic UCLP using cone beam computed tomography (CBCT) for placement of mini-screws to protract the maxilla.

Methods: This was a retrospective study utilizing CBCT scans of individuals (age 9-13, n=20) with non-syndromic UCLP. Using Invivo5 (Anatomage) software, non-cleft BCP and cleft BCP were compared using 1mm vertical intervals from 4mm-10mm above the Cemento Enamel Junction (CEJ). The buccal furcation of the maxillary 1st molar was used as a landmark for measuring the BCP. Statistical analyses for means of all measurements were completed using a paired t-test and intraclass correlation.

Results: Significant differences in BCP thickness were discovered at 6mm, 8mm, and 10mm above the CEJ ($p < 0.05$). Values ranged from 0.85 ± 0.19 - 1.17 ± 0.28 at non-cleft side and 0.90 ± 0.23 - 1.37 ± 0.34 at cleft side. Intra-examiner and inter-examiner reliability showed high intraclass correlation ($r = 0.98$ and $r = 0.86$, respectively).

Conclusions: In all instances, mean thickness of the BCP were found to be greater at the cleft side. All values increased from 4mm above the CEJ toward the apex on both sides. The findings indicate that to ensure optimal outcomes of treatment, mini-screw implants for maxillary protraction should be placed at least 10mm above the CEJ on both the cleft and non-cleft sides of patients exhibiting Class III malocclusion.

Support: Department of Orofacial Sciences

#11 Title: Unlocking the potential of oral commensal bacteria in the fight against cancer

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Objective: The incidence of oral squamous cell carcinoma is increasing worldwide, and the 5-year survival rate for oral cancer has not improved in decades, underscoring the need for novel diagnostic and treatment approaches. Although, smoking, alcohol consumption, and HPV infection contribute to the pathogenesis of oral cancer, the mechanistic contributions of the oral microbiome to oral cancer have not been well explored yet merit investigation. Epidemiological data suggest an association between the oral microbiome and oral cancer, and this association is supported by clinical and experimental data. We've demonstrated specific microbial shifts (loss of streptococci species) are associated with transitions from health to oral cancer. Our project seeks to identify oral commensal bacteria that can influence OSCC cell properties and further identify the mechanisms responsible for these effects.

Methods: OSCC cells were challenged with commensal Streptococcal strains (1.0×10^8 cells of *S. sanguinis*; ATCC 10556 and *S. mitis*; ATCC 10557) and evaluated for changes in OSCC cell proliferation, migration and integrin alpha V expression via Western Blotting. RNA-Seq was utilized to compare gene expression in OSCC cells challenged with and without bacteria. Data was analyzed for statistical significance using t-tests and ANOVA.

Results: *Streptococcus sanguinis* and, to a lesser degree, *Streptococcus mitis*, suppress OSCC carcinogenic properties, including cancer cell proliferation, migration, and integrin alpha v expression ($p < 0.05$). OSCC cells challenged with heat-deactivated strains display no inhibition in proliferation and migration, and OSCC cell migration was not affected by bacterial conditioned broth. RNA-seq data from *S. sanguinis*-challenged OSCC cells revealed a significant fold increase in tissue plasminogen activator, peptidase inhibitor 3, and Interleukin 8.

Conclusion: Our findings demonstrate that oral commensal bacteria, *S. sanguinis* and *S. mitis* mitigate OSCC carcinogenic properties. RNA-seq data identified differentially upregulated candidate genes in *S. sanguinis*-challenged OSCC cells that will be further examined regarding their potential in regulating anti-cancer effects.

Support: UCSF School of Dentistry Dean's Office

#12 Title: Characterizing phenotypic variation of craniofacial development in NOSIP-knockdown mice

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Objectives: Holoprosencephaly (HPE) is a disease exhibiting a spectrum of craniofacial malformations without a genotype-phenotype correlation. This study aims to characterize and analyze phenotypic variation using a mouse model of HPE with genetically altered Nitric Oxide Synthase Interacting Protein (NOSIP).

Methods: Embryos from a NOSIP-knockdown mouse model were collected at embryonic day 12.5 (E12.5), fixed, and stored in Paxgene. Embryos were imaged using confocal microscopy and 3-D imaged via micro-computed tomography. Embryos (n=3) were embedded in paraffin, sectioned at $10 \mu\text{m}$ and stained with H&E. RNA was extracted from the forebrain, midbrain, and maxillary and nasal processes of embryos (n=15) using a PaxGene RNA isolation kit. cDNA was synthesized from RNA, and gene expression analyses were performed using quantitative real-time PCR (qRT-PCR). Using GAPDH as a reference gene, the expression of *Axin2*, a downstream target of the canonical Wnt pathway, and *Patched* and *Gli*, components of Shh signaling, were analyzed. Changes in expression between wildtype (WT), heterozygous (HET) and homozygous knockout (KO) mice were compared using GraphPad Prism.

Results: Histological sections showed altered brain and face phenotype between WT and mutants, including a lack of separation between lateral outgrowth of the forebrain, abnormal eye development, and abnormal outgrowth of the maxillary processes without a mandibular process. Compared to the WT control, the HET and KO groups also exhibited a gradual trend of downregulation in both *Axin2* and *Gli* expression, indicating that NOSIP-knockdown groups had decreased activity of the canonical Wnt and Shh pathways.

Conclusions: NOSIP-knockdown results in phenotypic variation affecting the developing face and brain, suggesting NOSIP's crucial role in craniofacial development. Changes in Wnt and Shh signaling correlate with genotype and abnormal phenotype,

indicating that altered expression of these pathways is an important component contributing to the phenotypic variation exhibited by the NOSIP-knockdown mouse model of HPE.

Support: Contribution from Dr. Peter Sargent and Dr. Caroline Damsky, UCSF Faculty Emeritus; NIH R01DE019638

#13 Title: HPV16 E5 contributes to EGFR overexpression and activation in HNSCC

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Objective: A recent study showed that the HPV16 E5 oncogene plays a role in epidermal growth factor receptor (EGFR) overexpression in transformed HPV 16-positive anal epithelial cells (i.e. AKC2), however little research has been done looking at the role of E5 in HPV16-positive head and neck squamous cell carcinomas (HNSCC) cell lines. The goal of this research project was to detect E5 expression in HNSCC cell lines and investigate its role in EGFR overexpression and downstream EGFR signaling pathways.

Methods: HPV16 E5 expression was analyzed in two HNSCC cell lines (SCC90 and SCC47) using real-time PCR techniques. AKC2 cells were used as a positive control. SCC90 and AKC2 cells were transfected using E5-targeted siRNAs as well as negative control silent siRNAs. HPV16 E5 knockdown efficiency was evaluated 72-hours post transfection using real-time PCR. Cell proliferation was evaluated 72 hours post-transfection using standard cell counts. Total and phosphorylated EGFR and protein kinase B (AKT) protein expression were measured using western blot analysis and quantified using imageJ analysis.

Results: E5 RNA expression was 40- and 55-times higher in SCC90 and SCC47 cell lines, respectively, compared with AKC2 cells. Compared with SCC90 cells transfected with control siRNAs, transfection of SCC90 cells with E5-targeted siRNAs led to: 1) 85% reduction in E5 RNA expression; 2) 60% reduction in cell proliferation; 3) decreases in total EGFR and phosphorylated EGFR protein expression levels; and 4) decreased expression of phosphorylated AKT.

Conclusion: Our data show that similar to HPV 16-positive anal cell lines, E5 is expressed in HNSCC cell lines. E5 plays a role in EGFR overexpression and activation of downstream pathway proteins in HNSCC cells. Finally these data suggest that E5 could be a good therapeutic target for HNSCC.

Support: Contribution from Dr. John Greenspan and Dr. Deborah Greenspan, UCSF Faculty Emeritus; Helen Diller Family Comprehensive Cancer Center Support Grant

#14 Title: Characterization of RH3CC1 in the development and homeostasis of mouse dentition

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Objectives: Unlike humans, rodents have the ability to regenerate incisor enamel via stem cells housed in the labial cervical loop (laCL) region. We previously identified Rh3cc1 (R3H domain and coiled-coil containing 1) to be differentially expressed in the laCL during hibernation. The objectives of our study were to determine the expression of Rh3cc1 in adult and developing mice, and to analyze the effects of inhibiting Rh3cc1 on tooth development and incisor renewal.

Methods: To profile expression of Rh3cc1, RNAscope 2.5 Assay RED (Advanced Cell Diagnostic Bio) was performed following manufacturer's protocol at mouse embryonic (E) day 13.5, E15.5, E17.5, postnatal (P) day 7, and adult stages. To inhibit expression of Rh3cc1, timed-pregnant mice at E12.5 and E14.5 were injected with 10mg/kg of in vivo Rh3cc1 siRNA (Ambion). H&E staining and RNAscope for Rh3cc1 were performed.

Results: We detected Rh3cc1 expression expressed throughout the mouse body including the outer enamel epithelium (OEE) and inner enamel epithelium (IEE) of both developing mouse molars and incisors, as well as in the limbs and tail at E13.5 and E14.5. Starting from E15.5 and continuing to E18.5, stronger expression along the OEE and IEE was detected, and the signal became gradually stronger in ameloblasts. Stronger signal was observed in both cervical loops of molars. With inactivation of R3hcc1 using in vivo siRNA, we detected potential changes in dental epithelial stem cells (DESCs) in the adult laCL. Further analyses of adult laCL and offspring dentition are required.

Conclusion: Rh3cc1, a candidate gene identified to be involved in incisor renewal was dynamically expressed during tooth development and maintained expression in the adult laCL. The delivery of in vivo siRNA to timed-pregnant mice is a quick and efficient method to test the effects of Rh3cc1 during tooth development and incisor renewal.

Support: Department of Orofacial Sciences

#15 Title: Oral health-related quality of life in Cambodian children and adolescents

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Objectives: Few studies have investigated Cambodian oral health. This work-in-progress aims to describe changes in oral-health-related quality of life (OHRQoL) following intervention with oral hygiene instruction (OHI) to Cambodian children and adolescents ages 8-14 in the SF Bay and Stockton areas.

Methods: This randomized controlled trial includes two groups: 50 participants randomized to either control or OHI intervention groups. The Short Form Child Perceptions Questionnaire will be administered to both groups before the intervention. An immediate

post-intervention survey and follow-up surveys at 6- and 12-months will be administered to both groups. Differences in OHRQoL between pre- and post-questionnaires will be evaluated, as well as differences across the three post-questionnaires will be assessed with χ^2 test and tests for trends. To assess other factors that may have an effect on the bivariable comparisons between the two groups, multivariable analyses will be conducted, controlling for other demographic characteristics including gender, household income, parent's education, parent's immigration status, and SF Bay or Stockton area location.

Results: Currently, we have 24 participants, 16 boys and 8 girls ages 8-14 years old, in our study who have completed the pre-and immediate post-intervention surveys. We have amended our IRB to now include the SF Bay and Stockton areas and are working with 4 new partner organizations to recruit more children into our study.

Conclusions: Based on our interactions with the local Cambodian population this past year, we have learned that all our enrolled participants are first generation Cambodian American children, while almost all of their parents are born outside the U.S. It appears the parents are very traditional and are reluctant to accept outside influences, including dental resources. We have assembled a learning protocol from working with this unique community to provide future investigators with preliminary data for a better direction regarding future similar studies.

Support: Global Oral Health Community Partnership, 7 Partner Organizations (OCBTI, CCDI (SF Division), APSDRA, UKK, NDT, BICKBMC, and APSARA)

#16 Title: RUNX2 protein levels increase during embryonic development

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Objectives: Craniofacial abnormalities are the second most common birth defects, yet the underlying mechanisms linked to the pathogenesis are poorly understood. Many such defects affect the length of the jaw. We aim to elucidate a molecular mechanism regulating jaw length by studying Runx2, a key osteogenic transcriptional factor. Regulation of Runx2 has been linked to osteocyte-driven bone resorption by collagenases called matrix metalloproteinases (Mmps). We employ two models, quail and duck, which have distinct jaw lengths that can potentially be explained by differences in Runx2-mediated effects on Mmp13 expression and bone resorption. We hypothesize that Runx2 increases during late-stage development, and that there are species-specific differences in Runx2 and Mmp13, which could potentiate differences in jaw size.

Methods: Fertilized chicken, duck, and quail eggs were staged using the Hamburger-Hamilton (HH) system and mandibles were dissected at HH 31, 34, 37, and 40; then flash-frozen (n=9). Mandibles were homogenized in RIPA and quantified using BCA assay. Western blots, using 12% polyacrylamide gels and 40 μ g protein, were performed and probed using Runx2 (1:1000), Mmp13 (1:1000), and β -Actin (1:4000) antibodies. Bands, quantified using Image Studio, were normalized to β -Actin.

Results: Runx2 levels significantly increased ($p < 0.05$) HH37 and HH40 for all species. At late stages, chick had higher fold-changes in Runx2 when compared to similar stages in duck and quail. Mmp13 levels increased in all species with development, with quail having the highest levels and duck the least.

Conclusion: Runx2 and Mmp13 levels increased in all species as development progressed and was highest at stages 37 and 40, when bone resorption is initiated. Higher Runx2 and Mmp13 levels in chicken, compared to quail and duck, indicates a species-specific difference in bone resorption. This difference can explain variations in jaw length between species and can be the basis for future research through functional assays.

Support: NIH Grants R01DE025668 and R01DE016402.

#17 Title: PEGDMA microrods for localized and controlled release of NGF

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Objectives: Preliminary data from our lab has shown nerve growth factor (NGF) can improve fracture repair. The goal of this research is to develop an approach for controlled and localized release of NGF to the fracture callus via PEGDMA microrods.

Methods: 50 wt% PEGDMA microrods were fabricated via a photolithographic method using a precursor solution of PEGDMA placed on silicon wafers. Fabricated microrods were lyophilized and phospholipase A2 (a proxy molecule for NGF) was loaded into microrods via absorption for 24 hours. Loaded microrods were suspended in PBS and supernatant samples were taken twice every 24 hours. Samples were measured using a micro-BCA protein assay kit read at 562nm. A tibia fracture model of mice was used to determine microrod location in vivo. 500,000 microrods in 20uL of sterile PBS were injected into the callus 7 days post-fracture. Fracture calluses were harvested for histology and HBQ staining at 2, 7, and 14 days post-injection.

Results: Microrod synthesis was troubleshoot and optimized. HBQ staining did not show significant presence of microrods within the fracture callus, suggesting microrod migration from the injection site. After 2 days supernatant samples had a zero-level absorbance indicating burst release of total encapsulated protein by microrods.

Conclusions: The lack of viewable microrods histologically suggests migration from the fracture callus, possibly due to cellular turnover within the callus. It is also possible HBQ staining is inadequate for detection of microrods and a different localization method must be used. PEGDMA microrod's delayed release of PA2 indicates their potential as a delivery vehicle for NGF to the fracture callus. Further optimization of the microrod chemistry is necessary to tune release kinetics. Slower release of NGF/PA2 can

be accomplished by increasing the PEGDMA monomer to 75 wt% from 50 wt% in addition to using higher concentrations of PA2/NGF during loading.

Support: Department of Oral and Maxillofacial Surgery, Foundation of Orthopedic Trauma (FOT) Grant (Bahney), AO Foundation Career Development Awards (Bahney), Orthopedic Trauma Institute, Department of Bioengineering and Therapeutic Sciences

#18 Title: Characterization of KRT14+;KRT5+ progenitor cell function in adult murine salivary glands using non-adherent culture

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Objectives: The maintenance of progenitor cells is key in the formation and regeneration of functional salivary glands. Genetic lineage tracing studies determined Keratin 14 (K14+) and K5+ expressing cells are progenitor cells during fetal development of murine submandibular glands (SMGs).

Methods: To study the relationship between the K5+ and K14+ progenitor populations in the adult glands, we generated transgenic K14RfpK5Venus mice to fluorescently label K14+ with Rfp and K5+ cells with Venus. Both K14Rfp and K5Venus label the same cells in the adult SMG including myoepithelial cells, ducts, and small basal cells.

Results: We found that K14RfpK5Venus homozygous mice had reduced saliva flow and significantly smaller glands compared to the single transgenic and K14RfpK5Venus heterozygous (het) mice. K14Rfp SMGs had a significant reduction in the number of ducts and reduced gene expression of markers of myoepithelial (Acta2, Cnn1), progenitor (Krt14, Krt5, p63), and neuronal (Tubb3, Chat, Vip) cells compared to the wildtype, single transgenic K5Venus, and homozygous SMGs. This suggests overexpression of Rfp transgene in K14+ cells compromises their function, whereas K5Venus mice were normal. We dissociated SMGs from homozygous and het mice into single cells for salisphere assay. Their growth was analyzed using Celigo imaging cytometer at days 3 and 6 in floating culture. We found that homozygous salispheres were fewer in number and smaller but were maintained in culture, while there were more K5Venus salispheres that were larger than both homozygous and het salispheres. qPCR analysis of K14RfpK5Venus het salispheres showed reduced expression of myoepithelial markers, suggesting cell function was still compromised.

Conclusions: Overall, we developed a model to investigate functions of K5 and K14 cells in adult SMGs. We conclude that transgenic Rfp compromises myoepithelial function and may result in reduced salivary function. We will continue to study myoepithelial function and saliva secretion using the K5Venus line.

Support: NIDCR Summer Dental Student Award

#19 Title: Effect of dental curriculum hack-a-thon on student and faculty perception of dental education

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Objectives: There are numerous faculty vacancies for dental educators in the US. Dental educators come from various career paths: retiring clinicians, trainees who have completed post-graduate work, international dental graduates, and domestic dental graduates. In an effort to increase the latter pool, the American Dental Education Association (ADEA) has created a program called the Academic Dental Careers Fellowship Program (ADCFP) in which dental students interested in academic careers can learn more about that career path. Each fellow engages in a research or curriculum improvement project. In order to magnify the number of students interested in academic careers, one fellowship educational research project involved the creation of a Curriculum Hack-a-Thon, in which students from all six California dental schools were invited to learn about the complexities of creating curriculum and the contemporary issues facing dentistry. Faculty and administrators from these schools were invited to participate as judges.

Methods: A training session on how to develop curriculum and scoring rubric were provided to the students and faculty. Students and judges were administered a pre-event and post-event questionnaire to determine attendees knowledge on curriculum design, attitudes toward factors influencing dentistry and dental education, and beliefs about changes that need to be made to dental school curricula in the US.

Results: A total of 44 responses were received for the pre- and post-event questionnaire.

Conclusions: Participants reported an increase in knowledge of designing and implementing curriculum, the importance of a needs assessment, and the issues facing dentistry and dental education. Participants largely felt that the current model of dental education would not be sustainable, and that dental care delivery would continue to evolve. Participants felt that the drivers that would have the greatest impact to change dental education are: technology, cost of education, integrated models of healthcare delivery.

#20 Title: Tobacco industry involvement in periodontal research: a case study

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Objectives: Extensive evidence documents tobacco industry influence on medical organizations and research. In contrast, there is little understanding of how the tobacco industry may have influenced professional dental organizations and oral health research.

This study investigated possible reasons behind Philip Morris' (PM) inflation-adjusted \$750,000 in total contributions to the American Dental Association's Periodontal Pathology Research Center (ADA PPRC).

Methods: We searched the Truth Tobacco Industry Documents Library for internal tobacco industry documents from 1963 to 1973 that pertained to PM's contributions to the ADA PPRC. We used a snowball strategy to identify related documents, and used external sources to triangulate and contextualize findings. We then chronologically organized relevant documents into a narrative case study.

Results: As part of PM's diversification efforts, the company acquired Clark Bros Chewing Gum Company in 1963. PM's R&D focused on specialty chewing gums developed for anti-caries, anti-tartar, and other therapeutic functions. The primary reasons PM contributed to the ADA PPRC included attempts to expedite commercial development and to obtain the ADA Seal of Acceptance for its new dental products. Secondary reasons included being recognized as a source of dental research funding, acquiring 'de facto' periodontal/dental consultants, gaining positive brand name perception, and tracking new dental research using ADA connections. Importantly, PM's statements made to the public and the ADA regarding its intentions were incongruent with internal discussions on the topic. However, in 1973, PM sold Clark Gum due to unprofitability before commercial release of these dental product lines.

Conclusion: Philip Morris targeted the American Dental Association in order to gain influence not only for developing new products, but also for long-term benefits to both the Clark Gum and PM brands. This historical analysis illuminates the need for professional organizations to be conscious of ulterior reasons private corporations may have in developing partnerships.

Support: Delta Dental

#21 Title: High depression prevalence in dental students associated with lifestyle characteristics

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Objectives: Studies show health professional students have a high prevalence of depression. Dental students are especially susceptible due to the burden of the highest educational debt, exposure to bloodborne pathogens, and high frequency of chronic pain. However, few studies on depression amongst U.S. dental students exist. This study aims to determine (1) the difference in depression prevalence amongst U.S. dental students compared to that of the general population and medical students and (2) demographic and lifestyle characteristics associated with depression prevalence in dental students.

Methods: This cross-sectional study surveyed dental students in California using an anonymous 36-item Qualtrics™ online questionnaire. The Patient Health Questionnaire (PHQ-9), a recognized diagnostic screening tool, measured depression severity. Additionally, participants answered questions regarding demographic and lifestyle characteristics. Data analysis included chi-square tests for bivariable analyses.

Results: Investigators emailed invitations to 2552 domestic and international dental students, across all 4 years, in 6 California dental schools. The response rate was 21.4%. The crude depression prevalence (27.7%) was similar to that of U.S. medical students (27.2%) and higher than that of the general U.S. population (8.1%). Bivariable analysis found several demographic and lifestyle characteristics significantly associated ($p \leq 0.05$) with moderate to severe depression severity in dental students including: experience of injury/pain, lack of financial security, self-reported minority status, perception of meaningful work and year in school.

Conclusions: Prevalence of depression amongst California dental students is similar to that of U.S. medical students and higher than that of the general population. Results suggest that dental students are at high risk of experiencing mental health challenges and require accessible resources.

Support: ADEA ADCFP

#22 Title: Reliability testing of caries risk assessment tools utilizing a synthetic database

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Objectives: With the emergence of computerized clinical decision support in dentistry, there is lacking scientific evidence of comparison studies between two highly utilized tools: UCSF Caries Risk Assessment Clinical Decision Support (CRA CDS) and the leading commercially available caries risk determination tool, PreViser. This study aims to elucidate the correlation between CRA CDS and the PreViser tool in caries risk determination.

Methods: Factors affecting the caries risk were categorized into presence of dentin caries, previous restoration or extraction due to caries, heavy plaque, enamel lesions, dry mouth, and frequent snacking. These factors were mapped in CRA CDS and PreViser. A synthetic database containing all 64 possible scenarios was generated from CRA CDS variables. This 64 scenarios database was matched with PreViser variables and expanded to 4096 total caries risk assessments with PreViser variables. Intraclass correlation analysis was performed to determine two tools' agreement on a four-point scale risk level (very low/low, moderate, high, very high/extreme). Prevalence and bias-adjusted kappa (PABAK) was done to analyze the two tools' agreement on synthetic scenarios of not at risk versus at risk (very low/low = not at risk; moderate, high, extreme/very high = at risk)

Results: The intraclass correlation coefficient for the two tools' agreement on the 4-point risk scale was 0.48 (CI 0.45-0.51), fair correlation. The PABAK for the agreement on risk versus not at risk was 0.85 with an agreement of 92%, excellent correlation.

Conclusion: CRA CDS and PreViser caries risk assessment have excellent agreement when determining caries risk. The two tools have fair agreement in determining the level of risk.

Support: Delta Dental and Summer Fellowship Training; Department of Preventive and Restorative Dental Sciences

#23 Title: Validation of novel anti-sense siRNAs in-vivo

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Objectives: Small interfering RNAs (siRNAs) encompass several classes of RNA species that act as critical regulators of gene expression. MicroRNA, the most well-known class, was originally discovered to regulate *C. elegans* vulva development and this class was later found to regulate diverse human genes ranging from anti-oncogenes such as P53 to oncogenes such as RAS. Recently, our lab discovered a new class of siRNA whose members map to sequences that are antisense of intronic regions of genes in *C. elegans* (aiRNA). Remarkably we found that aiRNA are produced in human breast cells. Further, in collaboration with Drs Matt Gruener, Andrei Goga and Hani Goodzari, we found that these small RNAs are upregulated by at least 3 fold in breast tumor tissues from both human and mice. Thus, this new class of small RNAs are conserved from nematodes to mammals. My project aims first to examine if this newly identified RNA species regulates gene expression in *C. elegans*. Next, I aim to ask if this class of RNA is increased in oral, head and neck cancers.

Methods: Analysis of predicted aiRNA targets were made using libraries generated by L'Etoile, Fire, Miska, and Ruvkun's labs. A corresponding analysis was made from libraries generated from normal and cancerous human and mouse tissues. 1. To determine if the aiRNA do indeed regulate expression of *C. elegans* genes, we identified three top candidates based on their expression levels and whether they were found in complex with Argonaute RNA binding proteins. We generated probes to quantitate their target mRNA expression levels and generated RNA "sponges" (TuDecoy) that are designed to sequester and inactivate these aiRNAs. 2. To determine if this class of aiRNA is amplified in oral head and neck cancer, we will generate and analyze small RNA libraries from normal and tumor tissues from oral and head and neck cancers from oral surgery.

Results: Our work is on-going and we will present the results of our analysis of the *C. elegans* aiRNA data. We will also present our data showing that mouse and human aiRNAs are increased in abundance in tumor tissues. We will also present the results of our validation of the tools we have generated so far.

Conclusions: The newly discovered class of small RNAs, the aiRNA are conserved in nematodes and mammals and may be upregulated in cancer. This may provide both a biomarker for cancer as well as a window into how biogenesis of this class of siRNA is related to oncogenic transformation, which in the future, could inform therapy.

Support: NCI U19

#24 Title: 'I don't need perfect': stakeholder perspectives of direct-to-consumer orthodontic companies

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Objectives: There is a large unmet need for orthodontic treatment in the United States. Direct-to-consumer orthodontic companies (DCOs) allow patients to receive orthodontic clear aligner treatment (CAT) through the mail at a fraction of the cost of traditional orthodontic treatment. This study aims to understand stakeholder perceptions towards this new orthodontic care delivery model.

Methods: Three groups of stakeholders were invited to participate: patients, traditional orthodontists, and DCO representatives. Patients were recruited through DCO consumer Facebook groups, orthodontists and representatives through email. Semi-structured interviews addressed experiences, motivations, advantages and perceived risk of using DCOs. Interviews were audio recorded, transcribed, and coded for common themes using a social constructionist approach with Dedoose software.

Results: 7 orthodontists and 10 patients were interviewed. Despite multiple invitations and follow-up communications, no companies chose to participate. Orthodontists acknowledged the competitive prices offered by DCOs, but raised concerns that DCOs provide a lower standard of care and indicated a need to improve patient education regarding orthodontics and DCOs. Patients chose DCOs for the low prices. They primarily initiated orthodontic treatment for cosmetic reasons and communicated they were not looking for perfection, simply straighter teeth. Online communities were used as a resource to inform treatment decisions and manage care. Patients received warnings about DCOs from dental professionals, and usually sought in-office consultations with their dentist and/or an orthodontist prior to initiating DCO care.

Conclusion: Both orthodontists and patients cite lower cost as the largest factor in selecting a DCO over traditional orthodontics. Patients are aware of some of the risks of DCOs but attempted to avoid them through seeking an in-office visit with their dentist and using online support groups. This study is the first to examine the presence of DCOs and the results will help inform stakeholders and future studies evaluating this orthodontic care delivery model.

Support: Buchanan Dental Clinic

#25 Title: Multispectral imaging of enamel demineralization

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Objective: Most new lesions are found in the pits and fissures of occlusal tooth surfaces. However, conventional techniques, namely dental x-rays, do not provide high sensitivity for detecting early occlusal lesions. Furthermore, studies have shown that

visible- and fluorescence-based caries detection systems suffer false positives from staining. Previous work has demonstrated that reflectance imaging at 1450-nm yields high contrast between sound and lesion structure without the interference from staining. However, it is not known whether longer NIR wavelengths demonstrate higher lesion contrast and if lesion severity can be determined from multiple wavelengths. Our objective is to determine which NIR wavelengths for reflectance imaging yields the highest lesion contrast and if its multispectral analysis can infer lesion depth and severity.

Methods: For this study, (n = 25) human molars were collected and sterilized. Acid-resistant varnish was applied over occlusal surfaces excluding a 1.5 x 1.5-mm window. This window was exposed for varying degrees of demineralization: 24, 48, 72, 96, and 120 hours; with a surface softened lesion model at a pH of 4.5. Tooth samples were imaged at selected wavelengths from 405 – 1950-nm. Contrast measurements were calculated using windows of demineralization and sound tissue.

Results: Highest contrast was observed at wavelengths beyond 1460-nm. The contrast was significantly higher (P< 0.05) at 1950-nm than other wavelengths. Also, the variation in contrast increases significantly with increasing wavelengths with blue light having the lowest variation and NIR-REF the highest.

Conclusion: This study demonstrates that NIR reflectance at 1950-nm can provide high contrast images of dental lesions for early detection of occlusal dental lesions, and that contrast ratio increases with lesion depth at 1950-nm.

Support: Delta Dental, and NIH/NIDCR grants ROI-DE14698.

#26 Title: The roles of SATB1 on the morphogenesis of secretory ameloblasts

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Objectives: Enamel formation starts with depositing the full thickness of organic enamel matrix proteins into enamel space by secretory ameloblasts (SABs) through their apical surface. Special AT-rich sequence-binding protein 1 (SATB1), a higher-order chromatin organizer, was found to be significantly upregulated in presecretory ameloblasts, then downregulated in SABs. Understanding the molecular mechanisms of SATB1 that contribute to secretory ameloblast differentiation allow scientists to someday be able to regenerate enamel-forming ameloblasts.

Methods: The enamel density and enamel rod structure of P13 *Satb1*^{+/+} and *Satb1*^{-/-} mouse teeth were analyzed through X-ray microCT imaging and scanning electron microscope. Hemimandibles from various age of *Satb1*^{+/+} and *Satb1*^{-/-} mice were dissected, paraffin-embedded and processed for histological and immunochemistry analyses, and actin filament labeling to determine the effects of SATB1 on ameloblast polarity, actin filament assembly and secretory vesicle trafficking.

Results: In the mice with the deletion of *Satb1* gene, poorly mineralized enamel was developed and enamel rods were disorganized. Secretory ameloblasts (SABs) in *Satb1*^{-/-} mice were significantly shorter in length and lost the cytoplasmic protrusion Tomes' processes and terminal actin filament band that appeared at their apical pole of wild type (wt) SABs. A majority of amelogenins remained in *Satb1*^{-/-} SABs instead of depositing to enamel matrix, resulting in a thin, hypomineralized and non-prismatic enamel.

Conclusion: Through regulating the expression of a set of genes associated with SAB apical polarity, F-actin assembly and vesicle packaging, SATB1 is critical for ameloblastic vesicle trafficking to enamel matrix space. This study suggests the roles of SATB1 on ameloblast morphogenesis and its potential application in functional ameloblast regeneration.

Support: Department of Orofacial Sciences, NIH/NIDCR R03DE019507, R01DE027076 to YZ

#27 Title: Patient-reported adverse events and near misses at the dental office

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Objective: In dentistry, very little research has been done on patient-reported safety events. The purpose of this study was to assess the ability of dental patients to identify adverse events (AEs) and near misses in the dental office, the characteristics of these events, and their severities.

Methods: We conducted an observational study with adult dental patients at the UCSF Dental Center over a five-week study period. Using a semi-structured interview questionnaire, patients were contacted one to four weeks following their index dental visit via telephone. Patient responses were categorized according to AE Type, AE Severity, and Quality of Care (QOC) issues. The final determination was made using a consensus process between two dental safety experts. Chart reviews were conducted to confirm patient reports and/or identify unreported events.

Results: Of the 315 patients enrolled in the study, 231 (73.3%) completed the follow-up telephone interviews. Majority of participants were female (54%), white (52%), college-educated (59%), and aged 45 to 64 years (40%). Thirty-five participants (15.1%) reported experiencing an AE, and no patient reported a near miss. Most of the patient-reported AEs (80%) were pain-related, and rated an E1 on the AE severity scale. 75% of the patient-reported AEs were not documented in the EHR. Conversely, only one AE identified through the chart review process, was not reported by patients. 26% of patients reported poor QOC experiences, such as mismatched treatment expectations, financial disputes, and miscommunication.

Conclusion: Patients are able to identify safety events, when prompted appropriately. Their willingness to report these events often depend on its severity, thereby, creating missed learning opportunities. Poor QOC experiences are often regarded as AEs.

Support: The John C. Greene Fund

#28 Title: Peripheral and central effects of Botox on post-traumatic stress disorder of patients being treated for temporomandibular joint disorder

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Objectives: Chronic pain is the most common cause of long-term disability in the United States, yet the underlying mechanisms are poorly understood. Post-Traumatic Stress Disorder (PTSD) and Temporomandibular Joint Disorder (TMD) are two chronic pain disorders that often present concurrently, especially in our veteran population. Incobotulinum toxin (Botox) injections are a widely used treatment for TMD, yet injections have also been seen to relieve PTSD symptoms, such as anxiety and sleep disorders. This study aims to determine the effects that Botox is having on the central nervous system via changes in peripheral nociceptive (afferent) pathways, allowing the injections to relieve symptoms of both disorders.

Methods: The study is double-blinded, randomized and placebo-controlled. There are currently 7 patients enrolled in the study that met requirements, with previous diagnoses of TMD and PTSD. The study involves an initial and follow-up appointment (6 weeks). Both appointments include an fMRI of the brain, as well as, an ultrasound and electromyogram of the muscles of mastication. At the initial appointment, patients are given injections into bilateral masseter and temporalis muscles of either Botox or saline (placebo). The patients are asked to fill out a pain application, daily, which monitors pain and function between appointments.

Progress Report: Comparing average pain prior to injections and after, one patient reports a significant decrease from an initial 5.5 to 3.5 (scale of 1-10), while another patient reports no change in pain.

Future Directions: The study will be un-blinded after 10 patients have received the full treatment. It is expected that the results will show changes in both central and peripheral pathways, as well as, a decrease in muscle thickness and activity in patients that received the Botox injections.

Support: San Francisco Veteran Affairs Medical Center

#29 Title: Sugar industry's use of dietitians to influence dental professionals, 1974-1978

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Objectives: In 1975, the U.S. Sugar Association (SA) created the Regional Nutritional Information Program (RNIP) with the goal of enlisting dietitians to spread positive messages about sugar and health. The objective of this study was to describe the RNIP, and determine its impact on dental professionals.

Methods: 304 internal SA documents from 1974 to 1978 were reviewed for relevance to the RNIP. 59 documents were identified for further review and emerging themes were identified. Secondary sources were used to contextualize findings.

Results: The RNIP was an essential component of SA's public relations campaign designed to portray the safety and benefits of sugar in a balanced diet. SA launched the RNIP in 1975 with one home economist that traveled to Utah, Idaho, Colorado, Texas and, New Mexico. In 1976, SA had hired 8 dietitians in Hartford/Springfield, Columbus, San Diego, Albuquerque, Dallas/ Ft. Worth, Oklahoma City, Kansas City and Nashville. The RNIP's target audiences included universities, public schools, professional associations, and the media, among others. SA's dietitians hosted educational workshops, incorporated SA literature into libraries and curricula, monitored professional and consumer attitudes toward sugar, and made media appearances. By mid-1978, SA's dietitians had traveled to 110 cities in 33 states and appeared on 251 TV and radio programs. SA's dietitians interacted with dental professionals by: documenting their attitudes toward sugar, influencing a professional conference to include pro-sugar speakers, developing a media program that minimized sugar's role in tooth decay, monitoring and attempting to persuade Dr. Ira Shannon a dentist-researcher examining the high-sugar content of breakfast cereals, and sending comments critical of his work to newspaper editors.

Conclusion: SA used the RNIP to balance dentist's anti-sugar messages at professional meetings and in the media. The public health community should consider corporate relationships in the dietetic profession as a potential determinant of oral health

Support: Program in Global Oral Health, The Laura and John Arnold Foundation

#30 Title: Mimicking a sinus lift procedure in a murine model

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Objectives: Sinus lift surgeries, required prior to the placement of a maxillary dental implant in cases of severe alveolar bone loss, delay the implant placement for up to 6 months. Advances in stem cell biology offer a wide range of applications in dental surgery, all aimed at improving the healing process. Several animal species have been used as preclinical models for maxillary sinus lift

surgeries, namely sheep and rabbits, but only mice are suitable for transgenic strains and could allow the study of molecular pathways of the healing processes. We postulated that despite anatomical discrepancies with a human maxillary sinus and the small size of the surgical site, mice would provide a suitable preclinical model for this procedure

Methods: After maxillary first molar extraction and successive socket healing, a mucosal tissue flap was elevated at the extraction site. Alveolar bone was drilled until the nasal cavity membrane was visible. Bone chips from either xenogenic origin (Bio-Oss, SUI) or harvested from syngenic GFP mice were placed under the lifted membrane and the surgical site was closed using a collagenous membrane (Bio-Guide, SUI).

Results: Qualitative measures were used to analyze the outcomes of the surgeries. Microcomputed tomography analysis revealed proper bone chip placement above the maxillary molar extraction site. Staining with Movat's pentachrome stain and aniline blue revealed active osteogenesis around the bone chips. Immunohistochemistry involving the GFP-labelled graft showed – for the first time – the relative contribution of the host to transplanted cells in bone healing and formation in the sinus.

Conclusions: Our data demonstrate that a murine model can be used to study sinus lift surgeries. Further testing of bone regenerative technologies using this model would provide new insights into therapeutics that could accelerate bone volume augmentation prior to implant surgery.

Support: Stanford University Division of Plastic and Reconstructive Surgery

#31 Title: Sensor to induce and quantify interproximal forces during tooth movement

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Objectives: Preliminary research conducted at UCSF utilized a modified "Waldo Method" to induce experimental tooth movement (ETM) using an elastic spacer placed interproximally between mice molars. In this proof-of-concept study, the first objective was to replace the spacer with a capacitive sensor to induce and quantify the real-time force on the tooth-crown. The second objective was to relate the force on the crown to changes in the PDL-space. Experiments were performed on a rabbit model in situ for correlation of measured forces on the crown with displacement of the tooth-root within the dentoalveolar complex.

Methods: The effect of change in sensor capacitance on change in PDL-space width was investigated by using sensors of thicknesses 60%, 20% and 0% greater than PDL-space. Sensors were placed interproximally between mandibular molars of freshly harvested rabbits. Shifts in capacitance values were recorded by the sensor, and spatial associations of teeth relative to their respective bony sockets were mapped using X-ray micro-computed tomography (micro-XCT) scans.

Results: For a 70% overall increase in capacitance, 60% widening and 30% narrowing of the PDL-space in the experimental group were observed. Altered PDL-spaces also were observed for a 40% increase in sensor capacitance, with 16% widening and 10% narrowing. However, it is likely that the time-dependent component of the PDL could dominate and the intended long-term effect of the 40% capacitance increase could be minimal toward tooth movement.

Conclusion: This proof-of-concept data illustrated that the initial effect of 40% increase in capacitance on tooth movement could be masked by the viscoelastic effects of the PDL. However, a 70% capacitance increase could prompt significant tooth translation. Tuning capacitance and thereby force on the tooth-crown and correlating these adjustments with changes in PDL-space could provide insights into mechanistic processes within the periodontal complex.

Support: 1R21DE027138-01. Department of Preventive and Restorative Dental Sciences, UCSF School of Dentistry. Department of Mechanical Engineering, Stanford University.

#32 Title: Rater variability in clinical general dentistry competency exams

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UCSF School of Dentistry

Objectives: Faculty calibration is a top concern among graduating dental students across the nation, as captured by the American Dental Education Association (ADEA) Graduate Survey. This concern is reverberated from students and faculty at the UCSF School of Dentistry, however data analysis to confirm that this is a true problem has not been completed. This study aims to evaluate variability among examiner scores on three clinical competency exams given in the fourth-year.

Methods: Scores from Class of 2017 fourth year clinical competency exams in Oral Diagnosis and Treatment Planning (ODTP), Class II and Class III/IV Restorative were collected. The following information was extracted from exam forms: student, examiner, score for 15 factors and overall score for ODTP, score for 7 factors and overall score for Class II and Class III/IV. Forms with incomplete data were not used. Examiner gender, student gender, student coaching group, and student enrollment in the domestic (DDS) or international (IDP) pathways were recorded.

Results: A total of 98 ODTP and 262 Restorative exams were recorded. For ODTP exams, the mean overall score was 30 out of 35 with a standard deviation of 3.53; for restorative exams, the mean overall score was 54.5 with a standard deviation of 5. No significant difference was found based on status as DDS vs. IDP. An association was found between student and examiner gender.

Conclusion: The data shows that overall scores for exams are within a narrow range. The association between student gender and examiner would benefit from further analysis.

Support: Unfunded project through the Academic Dental Careers Fellowship Pathway

#33 Title: The effects of a short training session on the quantity of item writing flaws

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UCSF School of Dentistry

Objectives: Multiple Choice Questions (MCQ's) are widely used for testing in health sciences education and require careful development to ensure fairness and validity. Faculty that are inadequately trained to write MCQ's commonly produce poor quality, flawed, or low-cognitive-level items. Exams with flawed items are less reliable, more difficult and less discriminating. The purpose of this study is to evaluate how a short 30-minute training session on common Item Writing Flaws (IWFs) given to UCSF School of Dentistry faculty will have an affect on the quantity of IWFs relative to faculty that have not received training.

Methods: 23 UCSF Faculty members were randomly placed into 2 groups and asked to submit 6 MCQ each. The control group (11) was asked to improve their questions without direction. The experimental group (12) was given a 30-minute training session on common IWFs and asked to improve their questions. The rater looked for each of the 8 item flaws presented in the training session in every question (pre and post-intervention).

Results: The Intervention group had a 45% decrease in the total number of IWFs between Pre- and Post-Intervention, while the Non-intervention group only had a 10% decrease.

Conclusions: The study showed no significant difference between the pre-test comparisons of the control and experimental Groups ($p=0.80$); however, the post-test results were statistically significant ($p=0.004$). This study provides evidence that a short training session requiring minimal resources is effective in reducing the number of Item Writing Flaws of in-house MCQ's among dental school faculty. Future studies involving multiple dental schools may validate our initial findings.

Support: ADCFP

#34 Title: Outreach program for establishing early pipeline of diverse dental workforce

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Objectives: One potential solution for reducing oral health disparities is to increase more underrepresented minority (URM) populations into dental workforce. This study examined the preliminary efficacy of College Day, an annual one-day outreach event for URM, low-income undergraduate students attending colleges in California. The purpose of College Day is to expose URM students to the dental profession and various related topics, such as the dental school admission process, loan financing and repayment programs, and public dental health issues.

Methods: A pre- and post-study design using a survey to assess oral-health related knowledge, confidence, attitude, and intent to serve the oral health needs in underserved communities was utilized. Sociodemographic data, including age, gender, and race were also collected. Changes between pre/post-responses were analyzed with paired-sample t-test. Differences in outcomes by gender, race, and income levels were analyzed with independent-samples t-test and ANOVA.

Results: A total of 109 college students participated in College Day from 2016 to 2018, of which 91(83.5%) filled both pre and post-surveys. Among those that completed surveys, 69% were female, 44% reported Asian, 77% were 20 years old and older, and 53% reported a family income of under \$50,000. Immediately following the event, there was an overall significant increase in participants' knowledge ($p=0.011$) and attitude ($p=0.010$) in serving vulnerable populations. There were no statistically significant differences in confidence and intent. There were also no statistically significant differences in outcomes by gender, race, and income levels.

Conclusion: A one day outreach event was effective in improving college students' knowledge on oral health disparities and attitude in serving the underserved communities regardless gender, race, and income levels. Outreach events targeting URM college students interested in a career in dentistry may be one way to increase workforce diversity to increase access to oral care in underserved populations.

Support: Lee Hysan Fund, United States Health Resources & Services Administration (HRSA) Grant No. D85HP28498

#35 Title: Stress and stress interventions in dental students' transition to clinic

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Objectives: This study aims to determine sources of stress for third-year dental students transitioning from pre-clinic to clinic, student ratings of potential interventions for transition improvement, and associated student characteristics.

Methods: A cross-sectional, 28-item online survey was emailed to 91 third-year dental students transitioning from pre-clinic to clinic. Students responded to a 5-category Likert scale to rate 21 stressors' degree of stress, and 8 proposed stress interventions' degree of usefulness in improving their clinical experience. The survey also evaluated student demographic and life-related

characteristics associated with the transition-related stress.

Statistical analyses included Chi-square tests and generalized models to evaluate associations between student characteristics and reported stressors, and evaluate correlations between stressors and interventions.

Results: Fifty-nine students completed the survey (response rate=65%). Top five stressors were long wait times for faculty; multitasking clinical and administrative needs during clinic sessions; and insufficient chair time, support from clinical faculty, and variety of clinical procedures. Top three interventions were moving year-3 rotations to year-2, basing clinical requirements on patient needs rather than procedures, and increasing clinic experiences in year-2. Statistically significant correlations ($p \leq 0.05$) existed between multitasking clinical and administrative needs and basing requirements based on patient needs rather than procedures, between long wait times for faculty and increased clinical experience in second year, and between long wait times for faculty and more third year rotations in second year. Student characteristics significantly associated with stress included race/ethnicity, other life-related stressors, and additional graduate degrees.

Conclusion: The study identified stressors and interventions that students rated highly on a 5-point Likert scale. Some significant correlations existed between the highest ranked stressors and interventions. The study's results may help dental schools plan interventions for students transitioning to clinic to reduce students' stress and improve clinic experience.

Support: Department of Preventive and Restorative Dental Sciences, UCSF School of Dentistry

#36 Title: Correlation of PRX1 and SCX biomolecules at dentoalveolar joint ligament-entheses

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Objectives: Understanding tissue regenerative capacity of the enthelial regions in dentoalveolar joint (DAJ) will aid in optimizing orthodontic tooth movement and implant osseointegration. Paired-related homeobox protein1 (Prx1) expressing cells, denoting an osteochondroprogenitor, is minimally characterized at the DAJ. This study seeks to determine if Prx1-positive stem cells populate the Scleraxis(Scx)-positive mechanoresponsive ligament-entheses of the DAJ. Specifically, correlative mapping of osteogenic marker osterix (Osx) to Prx1 and Scx expressions at mineral forming and resorbing ligament-enthelial regions of the periodontal complex will reveal the relationship between fibrogenesis and osteogenesis in the DAJ.

Materials/Methods: Transgenic Prx1-CreER mice (N=3, 3-month old) were marked with red tdTomato (tdT) fluorescence for lineage tracing after 5-consecutive-days of tamoxifen injections. Green fluorescence signals of Scx-positive cells from ScxGFP-transgenic reporter mouse (1-month old) were mapped to identify mechanically active regions within the periodontal complex. Immunolabelling for Osx was performed and its expression was correlated with Prx1 and Scx expression in fluorescence microscopy. Subsequently, fluorescent labels were correlated with structural features of the ligament-entheses using a field emission electron microscopy technique.

Results: DAJ Prx1 expression scattered within incisor root pulp, perivascular regions within incisal periodontal ligament (PDL), and the surrounding alveolar bone periosteum with higher levels at PDL-bone entheses. There was no detectable Prx1 expression at the molar roots. In 3-months old Prx1-CreER mice, Osx expression co-localized with PDL vasculature proteins. Strong Scx expression levels spanned across PDL. In ScxGFP 1-month old mice, Osx expression within PDL dominated at PDL-bone and less so at PDL-cementum entheses, with no Scx overlap at both ligament-entheses.

Conclusion: No correlation was substantiated between Prx1 and Scx expressions. Prx1 and osteogenic Osx marker localizations are tissue-specific with Osx activity likely exhibiting age-dependence. Results compel a need to perform an age-related baseline study to map osteogenic stem cell biomolecular expressions within the DAJ.

Support: Buchanan Dental Clinic

#37 Title: Oral health and nutrition knowledge and practices, and dental care: a focus group study in rural Nepal

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Methods: This qualitative study took place in four rural regions of Parbat and Kaski Districts, Nepal. Trained Nepali health workers led seven one-hour focus groups with 78 participants including mothers and other community members, community health workers, and government workers. The discussions addressed nutrition and oral health knowledge and practices, and access to dental care. Discussions were audiotaped, translated and transcribed, and analyzed using Grounded Theory methodology, and oral health and nutrition themes were identified.

Results: Changes were noted from previous generations with increased knowledge of the importance of oral hygiene, yet limited knowledge on the pathophysiology of dental decay. School toothbrushing programs were considered helpful, however children's dietary shifts from traditional foods to frequent consumption of sweets posed a major challenge to maintaining good nutrition and oral health. A discrepancy between oral health and nutrition knowledge and practices was acknowledged. Access to dental treatment was noted as a major challenge and dental treatment was primarily utilized only for emergency care and treatment of severe pain. Barriers included busy work schedules, time-consuming and costly travel to urban clinics, high cost of dental care, variable quality of care, mistrust of dental providers, and myths of diseases or conditions caused by dental treatment.

Conclusions: Across focus groups, participants identified the need for oral hygiene and nutrition education programs. Although

basic knowledge exists, more emphasis is needed on prevention of oral disease. Participants called for government programs to improve access to dental care and facilitate positive community beliefs regarding preventive oral health care.

Support: Global Oral Health Research Fellowship 2017-2018

II. GRADUATE CATEGORY

#38 Title: Dehydration rate and transparent surface layer thickness for coronal lesions on extracted teeth

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Objectives: Transparent remineralized surface zones found on natural caries lesions may reduce the permeability to water and plaque generated acids. Near-IR (NIR) reflectance imaging coupled with dehydration can be used to measure changes in the fluid permeability of lesions in enamel and dentin. Previous work demonstrated a negative association between the surface zone thickness and the rate of dehydration in simulated enamel lesions. In this study, the rates of dehydration and thickness of transparent surface layer of coronal lesions of extracted teeth were measured and correlated.

Methods: Fifty extracted human premolar and molars with suspected coronal lesions determined by a clinician were selected for reflectance dehydration imaging at NIR wavelengths from 1695 nm. In addition, Optical Coherence Tomography (OCT) images of these enamel lesions were taken using 9x9 mm Microelectro-Mechanical System (MEMS) scanner. Dehydration results were correlated to transparent surface layer thickness using custom image processing and statistical software.

Results: There is a significant negative linear correlation between dehydration rate and transparent surface layer thickness ($r=-0.49$, $p=0.0004$).

Conclusion: There is a relationship between the transparent surface layer thickness and the lesion permeability. Small changes in thickness of $<50\mu\text{m}$ seemed to elicit larger changes in permeability. Histology will be performed to validate the results here.

Support: NIH/NIDCR Grant R01-DE14698, Grant F30-DE027264-02 Individual Predoctoral NRSA Fellowship Swiss National Science Foundation (SNF) AXSUN Technologies for the use of MEMS OCT System

#39 Title: *T. denticola* activates a TLR/Calcium-dependent pathway upstream of MMPs 11 and 28 in human periodontal ligament cells

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Objective: Patients who suffer from aggressive periodontal disease (PD) respond poorly to conventional treatment compared to patients with early or moderate PD. Even after clinical intervention, patients with aggressive PD still have a chronic pro-inflammatory transcriptional profile throughout the periodontium, leading to tissue-destruction and tooth loss. This differential response to treatment elucidates that: 1) pathogenic bacteria of aggressive PD may influence the host transcriptome and epigenome in order to mediate a suitable environment for survival; and 2) targeting whole pathways may be a more viable approach to treating PD progression rather than a single molecule. The oral spirochete, *Treponema denticola* (*T. denticola*) has been associated with aggressive PD. However, few direct links have been reported between *T. denticola* and the cellular/tissue processes that drive periodontal tissue destruction. This study aimed to explore the influence of *T. denticola* on host transcriptional profiles in human periodontal ligament cells (hPDL) by total RNA extraction and sequencing.

Methods: We challenged cultured human periodontal ligament (hPDL) cells with *T. denticola* bacteria followed by extraction and sequencing of host total RNA. Unchallenged hPDL cells were used as a negative control. Differential gene expression, gene ontology and KEGG pathway enrichment analysis were used to analyze data.

Results: MMPs 11 and 28 were amongst the top upregulated genes by *T. denticola* (both of which have not been characterized in the context of PD). Pathway enrichment analysis revealed Toll-Like Receptor, Calcium and MAP Kinase signaling to be enriched upon challenge. Downstream of these pathways, transcription factor, NFATC2, was upregulated ~ 2.95 fold.

Conclusion: We propose that *T. denticola* may activate a TLR2/Calcium-dependent pathway, activating transcription factor, NFATC2, which differentially regulates MMPs 11 and 28 in hPDL cells.

Support: NIH R01 DE025225 and NIH T32 #2T32DE007306

#40 Title: Antibiotic prescribing trends in periodontal surgeries with and without bone grafting

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Background: The prevention of post-operative infection following periodontal surgery is often the basis for antibiotic prescription. The inherent risks of unwarranted antibiotic use and lack of guidelines for procedures involving bone grafts creates an additional difficulty in decision making for practitioners. This study aims to evaluate trends in antibiotic prescribing for periodontal surgeries with and without bone grafting.

Methods: A validated, 15-question anonymous survey using Qualtrics software was distributed to American periodontists via email. The survey included questions about prescribing tendencies for specific periodontal procedures, rationale questions for choosing to prescribe or not to prescribe antibiotics, and demographic information. Results were analyzed using McNemar tests.

Results: 100 practitioners responded to the survey. Practitioners were significantly less likely to prescribe antibiotics for traditional periodontal surgeries involving no bone grafting compared to socket preservation, guided tissue regeneration, ridge augmentation and sinus augmentation ($p < 0.0001$). Practitioners were significantly more likely to prescribe antibiotics with more complex procedures involving bone grafting such as ridge augmentation and sinus augmentation when compared to socket preservation ($p < 0.0001$). 75% of practitioners responded that they would follow guidelines for antibiotic prescription with bone grafting procedures if they were developed and endorsed by the American Academy of Periodontology.

Conclusions: Practitioners are more likely to prescribe antibiotics when bone graft is used and as the complexity of the bone graft procedure increases. Based on these results and the desire for evidence-based guidelines, the establishment of guidelines for practitioners on the appropriate use of antibiotics would be of benefit to the dental profession.

Support: Global Oral Health Research Fellowship

#41 Title: The Role of WNT Signaling in early tooth development

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Objectives: Canonical Wnt signaling is evident throughout early mammalian tooth development, suggesting its important role during odontogenesis. However, the specific role of canonical Wnt signaling during early stages of tooth development remains unclear. Building on our lab's finding of Fgf8-enriched dental epithelial progenitor population at the initiation stage, this project aims to understand how canonical Wnt signaling regulates the early stages of molar invagination in mouse.

Materials/Methods: Wnt signaling pathway was conditionally hyperactivated or inactivated in dental epithelium using mouse genetics. The resulting phenotypes of Wnt Loss-of-function (WntLOF) and Wnt Gain-of-function (WntGOF) were analyzed at a molecular, cellular and tissue level.

Results: Hyperactivating Wnt signaling pathway led to evagination of the dental epithelium into the oral cavity, as opposed to the normally occurring invagination into the underlying mesenchyme. Conversely, invagination was completely prevented upon ablating Wnt signaling in dental epithelium. Several odontogenic gene expressions were reduced upon abrogation of Wnt signaling, while upregulated upon hyperactivation of Wnt pathway, suggesting the role of Wnt in modulating these genes. F-actin expression pattern in dental epithelia of WntLOF and WntGOF mutants suggests altered cell behavior of suprabasal cells that may drive the invagination. In addition, immunohistochemistry revealed altered expression of adhesion molecule E-cadherin and ECM component Collagen VI. Inhibition of collagen synthesis in WntGOF mandible could prevent evaginating phenotype in WntGOF. Interestingly, kidney capsule graft of evaginating structures from WntGOF gave rise to de novo teeth, suggesting invagination is not required for tooth development.

Conclusions: These findings indicate that proper Wnt signaling activity in dental epithelium is required for dental epithelial invagination.

Support: NIH/NIDCR

#42 Title: Cellular mechanisms of EPH/Ephrin mediated cell segregation and boundary formation in development

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Objectives: Cell segregation, or cellular self-organization, leads to boundary formation and is critical for the organization of morphogenetic movement and tissue patterning. Signaling between membrane bound ephrins and Eph receptor tyrosine kinases is essential in boundary formation, driving segregation between ephrin-expressing and Eph-expressing cells. Eph/Ephrin-driven boundary formation has been proposed to occur by repulsive migratory interactions that depend on bidirectional Eph/Ephrin signal transduction. Here we investigate the molecular and cellular mechanisms underlying Eph/ephrin-driven boundary formation.

Methods: Using a genetic in vivo model, mice that are mosaic for expression of X-linked ephrin-B1, and a cell culture system, we are able to interrogate the mechanisms underlying Eph/ephrin-driven cell segregation and boundary formation.

Results: Utilizing our mouse model and cell culture system we demonstrate that Eph/ephrin signaling results in cell segregation and the generation of a cortical actin differential, with increased cortical actin specifically in EphB2 expressing cells. Prevention of signaling through Rho-kinase (ROCK) disrupts cell segregation in vivo and in cell culture indicating that actomyosin contractility is critical for this process. Conversely, disruption of cadherin-mediated adhesion has little effect of self-organization. By analyzing the contact angle of cells we estimated the interfacial tension of EphB2-ephrinB1 heterotypic pairs relative to homotypic interactions. Contact is dramatically reduced in heterotypic pairs, indicating an increase in interfacial tension, and inhibitors of actomyosin contractility significantly diminish this increase. Additionally, using a 3D aggregate approach to observe multicellular scale organization we see that Eph/ephrin cell segregation affects large-scale tissue morphology dependent on actomyosin contractility.

Conclusions: These data suggest a model for cell segregation in which Eph/ephrin signaling results in a cortical actin differential that prevents cells from making stable contacts and drives cell segregation, and ultimately affects tissue morphology by modulating interfacial tension.

Support: NIH/NIDCR: R01DE023337 and R21DE025923 to J.O.B.

#43 Title: Understanding phenotypic variation of craniofacial diseases

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Objectives: Mutations are required for evolution to occur, but proper organismal development requires that they be buffered against. Although morphological variation occurs, developmental buffering only allows certain forms to appear with frequency, while intermediate forms are rare or do not exist. Recent studies suggest that non-linear relationships in development (between variables such as phenotype, gene dosage, cellular activity, etc) are a rich source of the buffering that underlies this disparity. Here, we test whether such nonlinearities underlie the highly variable phenotypes characterizing holoprosencephaly (HPE), wherein the forebrain and midface are underdeveloped. Mutations in Sonic hedgehog (SHH) signaling pathway members have been linked to HPE, but the source of phenotypic variation seen in patients is unknown. We use an allelic series of embryonic mice with discrete, genetic reductions in Shh and in low-density lipoprotein receptor-related protein (Lrp) 2 to test whether nonlinearities in SHH signaling underlie the HPE spectrum. Lrp2 is an endocytic receptor required for SHH-signaling activation in the developing forebrain, and Lrp2^{-/-} mice exhibit milder forms of HPE.

Methods: We use geometric morphometric analysis to quantify embryonic craniofacial shape variation, qPCR and in situ hybridization to measure SHH-signaling levels and spatial distribution, and TUNEL to examine cell death.

Results: Our data suggest that Lrp2^{-/-} and Lrp2^{-/-};Shh^{+/-} embryos exhibit a significantly more narrow upper jaw in comparison to other genotypes, as predicted, while there is no difference in craniofacial shape between the other genotypes. Interestingly, Lrp2^{-/-}; Shh^{+/-} and Lrp2^{-/-} embryos exhibit higher phenotypic variance than other genotypes. There is increased phenotypic variance in the Lrp2^{-/-}; Shh^{+/-} embryos than in Lrp2^{-/-} embryos, possibly due to mandibular asymmetry.

Conclusions: These findings suggest that loss of both functional Lrp2 alleles brings SHH signaling below a threshold level required for proper craniofacial development wherein small changes to gene expression result in highly variable phenotypes.

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#44 Title: Bond strength of glass ionomer cement to silver diamine fluoride treated caries

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Objectives: Silver diamine fluoride (SDF) is a caries arresting medicament that has numerous advantages in the comprehensive treatment of pediatric dental patients. Used in certain clinical applications, its capacity for arresting caries, remineralization, and antibacterial properties make it an ideal component of treatment plans. In large cavitated lesions, SDF can be used in conjunction with glass ionomer cement to combine the benefits of caries arrest and an interim therapeutic restoration. Very few studies have examined the effects of SDF on bond strength to carious lesions. This study measures shear bond strength of glass ionomer cement to dentin with artificial carious lesions.

Methods: The shear bond strength of glass ionomer cement to dentin with artificial carious lesions was tested with several variables. These included testing shear bond strength of glass ionomer cement to dentin with artificial carious lesions with/without the application of SDF, with/without use of conditioner, with varying amounts of time elapsed between SDF and glass ionomer, and over time.

Results: Results show no statistically significant change in microshear bond strength when SDF was applied, statistically significant increase in bond strength when conditioner was applied, and statistically significant increase in bond strength when one week elapsed between SDF application and glass ionomer placement.

Conclusions: Results suggest that in the clinical application of SDF, a stronger bond strength can be achieved with conditioner use and a one week time interval between SDF and glass ionomer placement.

Support: California Society of Pediatric Dentistry

#45 Title: Epithelial-mesenchymal signaling interactions required for timing of mandibular osteogenesis

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Objectives: Identifying molecular signals mediating the epithelial-mesenchymal interaction (EMI) would allow us to discover proteins that regulate mandibular osteogenesis, and which potentially could be used therapeutically to generate bone in cases of disease and injury.

Methods: An RNA-seq experiment was used to identify candidate genes involved in EMI in duck mandible. RNA in situ hybridization, Western blot and RT-qPCR were used to characterize the spatiotemporal expression of these genes qualitatively and quantitatively on the mRNA and protein levels in three avian species (duck, quail, and chicken). In vitro organ culture and tissue recombinations were used to test if expression of these genes in mandibular mesenchyme requires epithelial signaling. Gain-of-

function experiments tested if these genes regulate the timing of mandibular osteogenesis.

Results: Data from the RNA-seq experiment, RNA in situ, Western blot, and RT-qPCR (n=14) reveal that spatiotemporal changes in members of the WNT and CXC signaling pathways are present at the right time, place, and levels to mediate the osteogenic EMI in the mandible. In vitro organ cultures (n=7) confirmed expression of these genes in mandibular mesenchyme depends on epithelial signaling. Gain-of-function experiments showed mandibular osteogenesis is accelerated when CXC is overexpressed and the amount of osteoid formation significantly increases on the treated side compared to the non-treated side after 7 days of culture (n=5, p<0.05).

Conclusions: Novel epithelial factors, including WNTs and CXC, involved in timing of mandibular osteogenesis were identified. Especially, CXC has an osteogenic effect during EMI. This finding furthers our understanding of mechanisms underlying EMI in the mandible and leads to potentially novel therapeutic approaches to promote bone formation in the jaw following birth defects, disease, or injury.

Support: 1F30DE027616-01, R01DE016402, S10OD021664

#46 Title: Skeletal anabolism by concurrently targeting the PTH1 receptor and extracellular Ca²⁺ sensing receptor

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Objectives: Osteoporosis inflicts significant economic and personal health burdens and specifically triggers negative consequences for dental patients; e.g. tooth loss, implant failures, ill-fitting dentures, and osteonecrosis of the jaw. Intermittent injections of parathyroid hormone (iPTH) is the only FDA-approved osteoanabolic therapeutic for osteoporosis. Our recent studies have shown that increases in local [Ca²⁺] activate the extracellular Ca²⁺-sensing receptor (CaSR) in osteoblasts (OBs) to promote bone accrual and maturation. This project aims to determine whether calcemic effects of iPTH activate Osteoblast CaSRs to produce osteoanabolic responses.

Methods/Results: Injecting iPTH or calcimimetic alone produced hypercalcemia or hypocalcemia, respectively, 3 hrs after injections in wild-type (Wt) C57/B6 mice. Co-injections with calcimimetics normalized the Ca²⁺-elevating effects of iPTH. μ CT and histomorphometry analyses showed that PTH alone increased trabecular bone mass and thickness by \approx 8% (p<0.05) in distal femurs vs vehicle-injected controls. When calcimimetics were co-injected with PTH(1-34), there were robust increases in trabecular bone mass (\approx 21% or 2.5 fold over PTH treatment alone, p<0.05) and in thickness (\approx 17%, p<0.05). Co-injections of calcimimetic with iPTH also significantly increased cortical bone size, volume, and thickness at tibiofibular junction by 8-10% (p<0.01), and bone strength as indicated by a 30% increase in failure load (p<0.01) in 3-point bending tests, all which were absent with iPTH treatment alone. The inability of iPTH to produce anabolic effects on cortical bone was due to increases in local osteoclastic activities counteracting the modest bone-forming activities. In contrast, the bone-forming actions were much robust in the cortical bone of mice treated with combined iPTH/calcimimetic. In OB CaSR-KO mice, the osteoanabolic effects of combined iPTH/calcimimetic treatment was completely abrogated, supporting our hypothesis that OB CaSRs play an essential role in mediating skeletal responses to the treatment.

Conclusions: Our study has revealed novel synergistic actions of iPTH and extracellular Ca²⁺ in promoting skeletal anabolism.

Support: NIDCR F30DE024952

#47 Title: Effect of subgingival irrigation with 0.05% sodium hypochlorite as adjunct to SRP on subgingival microbiota and gingival inflammation in moderate to severe chronic periodontitis patients

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Objectives: This study aims to evaluate the use of sodium hypochlorite irrigation as adjunctive therapy to scaling and root planing for periodontal disease. The study focuses on the potential effects of sodium hypochlorite on the sub-gingival microbiological composition and clinical periodontal parameters.

Methods: This study is a single site, randomized, parallel clinical trial. The study focuses on patients diagnosed with moderate to severe periodontitis based on clinical examination in the UCSF Periodontology Clinic. Clinical periodontal parameters, including plaque index, bleeding on probing, probing pocket depth, and clinical attachment level, are measured at baseline and at 4-6 weeks. Subjects are randomly treated with either scaling and root planing with water irrigation (control) or scaling and root planing with 0.05% sodium hypochlorite irrigation. Sub-gingival microbial samples are collected from two sites per subject at baseline and at final evaluation at 4-6 weeks.

Results: This study is ongoing; therefore, initial results on clinical periodontal parameters and microbiota will be presented.

Conclusions: This study may provide further insight into the antimicrobial properties of sodium hypochlorite and its effect on the clinical parameters of periodontal disease. If it is found to be effective, sodium hypochlorite has the potential to be an affordable and accessible adjunct for patients with periodontal disease.

Support: UCSF Periodontology Clinic

#48 Title: Dental implant assessment and maintenance: attitudes and practices of dental hygienists in the U.S. - a national survey

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Background: Routine professional maintenance and clinical assessment are critical aspects of dental implant care. Dental hygienists play an important role in the detection and prevention of peri-implant tissue disease. CODA standards regarding implants have not been established for dental hygiene education programs. The literature lacks studies of dental hygienists regarding implant assessment and maintenance.

Aim: To investigate U.S. dental hygienists' attitudes and practices regarding assessment and maintenance of dental implants.

Materials and Methods: A 34-item quantitative, web-based survey was developed and distributed nationally to a randomly selected sample of 10,000 dental hygienists from the American Dental Hygienists' Association (ADHA) email database. All survey responses were analyzed and reported using frequency distributions.

Results: A total of 2033 dental hygienists (21%) responded. Nearly all the respondents (98%) who practiced dental hygiene also provided care to patients with dental implants. Many routinely assessed for bleeding/exudate, mobility, plaque/calculus, and tissue color around implants, however 34% rarely/never checked for cement around implants, 31% rarely/never probed, and 54% rarely/never checked the occlusion. Many respondents (44%) reported they were unable to remove plaque as well from implants as compared to teeth. A majority (60%) reported using plastic/resin scalers, however only 7% felt they were effective. While 5% reported using subgingival air-polishers, 71% felt they were effective. The most commonly recommended hygiene aid for patients with implants was an oral irrigator by 75% of the respondents. A majority (91%) reported continuing education courses as the primary source of implant-related knowledge. While a majority of dental hygienists reported providing care to patients with dental implants, there was variability in attitudes and practices among respondents regarding the assessment and maintenance care of implants. Curricula and continuing education focused on evidence-based implant care recommendations are needed, so patients receive the best care possible.

III. POSTDOCTORAL/RESEARCH SPECIALIST/VISITING SCHOLAR CATEGORY

#49 Title: Polymicrobial oral lavage induces bacterial colonization and bone destruction in a standard mouse model

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Objectives: Various mouse models of experimental periodontal disease have been used to evaluate treatments or to study disease processes. Compared to ligature models, oral lavage models are technically easier to handle and closely mimic the chronic exposure to periodontal pathogens seen in periodontal disease. The aim of this study was to develop a polymicrobial mouse model of periodontal disease in a standard genetic background that is inducible by oral lavage and that exhibits bacterial colonization and bone destruction.

Methods: 6 BALB/cByJ mice were randomly assigned to the infection (n=4) or the control groups (n=2). Porphyromonas gingivalis (Pg), Treponema denticola (Td), Tannerella forsythia (Tf), and Fusobacterium nucleatum (Fn) were cultured anaerobically and then mixed together for oral infection/lavage. After 7-day antibiotic treatment, the mice were received the polymicrobial inoculum by oral lavage over 4 consecutive days every week for 8 weeks. Then, oral swab samples were collected at 10 and 11 weeks for DNA extraction and PCR analyses to monitor bacterial colonization/infection. Mice were euthanized at 14 weeks and mandibles and left maxillae were resected and mechanically defleshed for evaluation of alveolar bone loss by morphometric analysis.

Results: Pg, Fn, and Td were all detected by PCR at 10 weeks but only Pg was detected at 11 weeks. Tf was not detected at either time point. Mice infected with the polymicrobial inoculum exhibited significantly more bone loss (0.26mm) than controls (0.16mm, P=0.023).

Conclusion: Pg, Fn, and Td successfully colonize the oral cavity of mice when administered by oral lavage. Tf may be more sensitive to aerobic conditions and oral lavage techniques than the other pathogens, since it was not detected in the oral swab samples. This polymicrobial oral lavage mouse model can be used to successfully induce periodontal bone destruction.

Support: AAP/Sunstar Innovation Grant; China Scholarship Council

#50 Title: Periodontal pathogens promote cancer aggressivity via integrin signaling that is bacteriocin responsive

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Objectives: Epidemiological studies reveal significant associations between periodontitis and oral cancer. However, knowledge about the contribution of periodontal pathogens to oral cancer and potential regulatory mechanisms involved is limited. Previously, we showed that nisin, a bacteriocin and commonly used food preservative, reduced oral cancer tumorigenesis and extended the life expectancy in tumor-bearing mice. Also, nisin has antimicrobial effects on key periodontal pathogens. Thus, the purpose of this

study was to test the hypothesis that key periodontal pathogens (*Porphyromonas gingivalis*, *Treponema denticola*, and *Fusobacterium nucleatum*) promote oral cancer via specific host-bacterial interactions, and nisin therapy may modulate these responses.

Methods: Pathogen effects on oral tumorigenesis were assessed by changes in apoptosis, proliferation, migration, invasion, tumorsphere assays, and xenograft mouse models. Since integrins are key regulators of cancer cell migration and tumorsphere formation, their mechanistic contributions on these processes were investigated. Nisin's potential modulatory effects on bacterially-mediated tumor promoting processes were further investigated.

Results: All three periodontal pathogens enhanced oral squamous cell carcinoma (OSCC) cell migration, invasion, stemness, and tumorigenesis *in vivo*, without significantly affecting cell proliferation or apoptosis. Importantly, oral commensal bacteria did not enhance OSCC cell migration. Pathogen-enhanced OSCC cell migration was mediated via integrin alpha V and FAK activation, since stably blocking alpha V or FAK expression abrogated these effects. Nisin inhibited these pathogen-mediated processes. Bepridil, a calcium channel blocker, inhibited nisin-mediated downregulation of alpha V. *T. denticola* lipooligosaccharide (LOS), *P. gingivalis* lipopolysaccharide (LPS) and *F. nucleatum* lipopolysaccharide promoted OSCC migration and enhanced alpha V expression.

Conclusion: In summary, periodontal pathogens via LOS/LPS promote stemness and migration of OSCC cells through integrin upregulation/FAK activation, and thereby contribute to a more aggressive cancer phenotype. A periodontal pathogen-rich environment negatively influences the tumor microenvironment. Nisin can modulate these pathogen-mediated effects, and thus has therapeutic potential as an antimicrobial and anti-tumorigenic agent.

Support: NIH; R01 DE025225 to YLK and JCF, IADR/GlaxoSmithKline Innovation in Oral Care Award to YLK and AAP-Sunstar Award to YLK.

#51 Title: Difference of artificial dental biofilm (co-culture) formation between on enamel surface and glass

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Objectives: to compare composition and structure of biofilm formed on the bovine enamel surface and glass surfaces in an *in vitro* artificial caries/biofilm model as well as their response to blue-light on treatment.

Methods: Biofilm with co-culture of *S. mutans* UA159 and *S. sanguinis* 10556 were formed either on bovine-enamel blocks or on glasses in an artificial caries model in Brain Heart Infusion Broth (BHI) containing 0.6% sucrose for 24 hours at 37°C facultatively. The biofilms were treated with blue-light at 1,173.5 mW/cm² for 3 minutes (accumulated fluences = 211J/cm²) with no blue-light treatment as control. Then, the biofilms were growth in BHI sucrose broth for another 24 hours. Planktonic pH, biofilm density, and biofilm bacteria levels by selective culture were recorded at 24 hours post-light treatment. The results between different surfaces and blue-light treatment were analyzed by Student t-tests using SPSS 10.

Results: Without blue-light treatment, the planktonic pH was significantly lower on enamel (4.5±0.1) than that on glass (5.1±0.1, P<.05) while no differences were found between substrates with blue-light treatment (P>.05). Biofilm OD was significantly higher on enamel group (0.05±0.00, 0.04±0.00, respectively) than on glass (0.01±0.00, 0.01±0.00, respectively) with or without blue-light treatment (P<.05). Without blue-light treatment, SM on enamel (8.09±0.08) was significantly higher than on glass (7.48±0.24, P<.05) but showed no differences on substrates with blue-light treatment, contrarily, SS on enamel (4.76±0.48 and 4.37±0.19, respectively) was significantly lower than on glass (5.39±0.34, 5.53±0.31, respectively) with or without blue-light treatment (P<.05). The SM/SS ratio were significantly lower on glass than on enamel with or without blue-light treatment (P<.05).

Conclusion: The biofilm formation of co-culture of *S. mutans* and *S. sanguinis* is affected by substrates used with enamel favoring *S. mutans* colonization and biofilm formation compared to glass. The substrate also affected biofilm responses to blue-light treatments.

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#52 Title: Optogenetic control of the +TIP complex to reveal local microtubule functions during neuronal morphogenesis.

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Objectives: Neuronal morphogenesis and maintenance relies strongly on a properly organized array of microtubules(MTs), which are thin cylinder-shaped polymers composed of tubulin. MTs form a structural scaffold inside cells, and serve as railroads that enable cargo delivery over long distances (>1m). MTs are important determinants of axonal pathfinding, which relies on the interpretation of guidance cues by the axonal growth cone, and while this involves MT stabilization in the direction of migration, the mechanistic role of MTs is unclear. To gain a better understanding of this process we set out to develop novel optogenetics tools that allow us to locally manipulate the microtubule cytoskeleton using light.

Methods: We recently developed a photo-inactivated (pi) variant of the key MT regulator EB1, that allowed us to optically control MT growth (van Haren et al. 2018, Nature Cell Biology). To implement this tool in neurons we have developed a genome editing strategy that enables us to generate pi-EB1 cells in a single step. We have applied this strategy to engineer pi-EB1 stem cells (pi-EB1_hiPS), which could subsequently be differentiated into cortical neurons.

Results: To generate pi-EB1_hiPSCs, we employed CRISPR to directly insert a genetically encoded photo-inactivation(pi) element into the WT EB1 locus. We obtained several homozygous pi-EB1_hiPS clones, in which we could control MT growth with blue light.

Differentiation of pi-EB1_hiPSCs to neurons allowed us to locally inactivate EB1 in various neuronal compartments, and by confining the growth of these neurons to adhesion-micropatterns we can now test how local MT dynamics control growth cone migration in a highly standardized manner.

Conclusion: We demonstrate the efficient generation of neurons with an optically controllable cytoskeleton via the targeted genomic insertion of a photo-inactivation element, which enables us to control the neuronal cytoskeleton with unmatched spatio-temporal resolution, and facilitates the study of neuronal polarization.

#53 Title: Comparison between antibacterial effect of photo activation disinfection and 0.12% chlorhexidine on cariogenic pathogens

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Objective: To compare the antibacterial effect of a photo activation disinfection (PAD) system (DENFOTEX) on *Streptococcus mutans* and *Lactobacillus acidophilus* in planktonic phase, biofilm, and dentin caries lesion to 0.12% chlorhexidine.

Methods: Overnight culture of *L. acidophilus* ATCC4356 in MRS broth and *S. mutans* ATCC25175 in Brain Heart Infusion (BHI) broth were re-suspended at 10^8 CFU/mL as planktonic bacteria. Biofilms on human dentin blocks in BHI or MRS broth supplemented with 1% sucrose anaerobically were formed at 37°C while dentinal caries formed on human dentine slabs similarly for 14 days. Bacterial suspensions, biofilms and dentin caries lesions were treated by either DENFOTEX at 500mW, 0.12% chlorhexidine, or saline (negative control) for 60s. After treatments, biofilm or dentinal caries bacteria were re-suspended by sonication and homogenizer, respectively. Then, all samples were serially diluted and enumerated on BHI or MRS plates after 72-hours anaerobic culture. Logarithmic colony forming units/ml of vital bacteria and percentages of viability using saline as 100% were calculated (n=12/group). Results were compared by ANOVA/Tukey's or Student T tests among groups.

Results: PDT and 0.12% chlorhexidine significantly reduced (p<0.05) the planktonic cell viability of *S. mutans*(46%, 52%) and *L. acidophilus*(61% , 50%). In biofilms, PDT exhibited better bacterial killing on *S. mutans* and *L. acidophilus*(19%, 48%) than chlorhexidine(9%, 40%, p<0.05). In dentinal caries, PDT and 0.12% chlorhexidine only showed minimal disinfection effects on *L. acidophilus* (13% and 17%, respectively, p <0.05) with no effects on *S. mutans* (<1% for both, p>0.05)

Conclusions: Both 0.12% chlorhexidine and PAD demonstrated antimicrobial effects against *S. mutans* and *L. acidophilus* in planktonic and biofilm phases but had minimal effects on dentinal caries lesions. PAD is an effective cavities disinfection device with limitation on dentinal lesions

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#54 Title: Nisin probiotic suppresses pathogenic oral biofilms and promotes healthier biofilm composition

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Objective: The oral microbiome is critical to maintaining oral health. Dysbiosis in the oral microbiome or within oral biofilms contributes to oral and systemic diseases. Uses of pre/probiotics have been proposed to re-establish a healthy microbiome. We previously reported that a prebiotic agent, nisin, has antibiofilm and antibacterial properties against oral pathogenic bacteria. Therefore, we tested the hypothesis that naturally occurring bacteriocins, such as nisin, and nisin-producing bacteria can serve as pre/probiotics to promote oral health and prevent oral disease using in vitro models.

Methods: 24 and 48 h human saliva-derived oral biofilms spiked with oral pathogenic bacteria (*Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Treponema denticola*) were used to test the biofilm modifying effects of nisin and nisin-producing bacterium, *Lactococcus lactis* (ATCC 11454). Changes in biofilm mass were evaluated by crystal violet staining. Changes in biofilm two-dimensional structure and viability were evaluated using the LIVE/DEAD BacLight Bacterial Viability Kit and confocal microscopy coupled with FIJI/Image J analysis. Changes in biofilm composition were evaluated by 16S RNA sequencing.

Results: Nisin significantly prevented biofilm formation at 24 and 48 h dose-dependently from 0.5 µg/mL to 2 µg/mL. Nisin also disrupted pre-existing biofilms at 24 and 48 h dose-dependently from 1 µg/mL to 50 µg/mL. Similarly, nisin-producing *L. lactis* prevented and disrupted biofilm formation dose-dependently at 24 and 48 h. Low doses of nisin (1 µg/mL) and nisin-producing *L. lactis* (1×10^3) significantly disrupted 48 h biofilms spiked with periodontal pathogens. Sequencing data showed that treatment of pathogen-spiked biofilms with nisin and nisin-producing *L. lactis* enhanced community diversity and richness, and significantly suppressed *Porphyromonas*, *Fusobacterium*, and *Aggregibacter* genera to levels at/below control/unspiked biofilms.

Conclusions: Nisin and nisin-producing *L. lactis* significantly suppress biofilm parameters, including the presence of pathogenic bacteria, thereby leading to "healthier" oral biofilms.

Support: AAP/Sunstar Innovation Grant; UCSF Summer Research Fellowship; CAPES Grant Number 88881.133124/2016-01

IV. RESEARCH ASSOCIATES

#55 Title: Translating the PILP mineralization method into restorative dentistry

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Objectives: To develop a method that allows the application of the polymer induced liquid precursor (PILP) method for the treatment of dental caries. Previous studies have indicated that using polyaspartic acid (pAsp) as PILP induces functional remineralization (FR) in demineralized dentin when used in solution. This study focuses on the transition from a strictly solution based laboratory application to a clinically delivery method of PILP mineralization in caries.

Methods: Artificial lesions approximately 140 micrometers in depth were created in a pH 5 acetic acid solution. These lesions were restored with commercial glass-ionomer cement A) BioCem (Resin-modified GIC, NuSmile) alone, B) BioCem with the addition of 40% pAsp, or C) BioCem rehydrated with highly concentrated pAsp solution. Each group contained three samples. The samples were then placed in (simulated body fluid) SBF at 37 degree Celsius on a 3-D rotating plate for two weeks prior to analysis. All samples were sectioned and analyzed using light microscopy for dehydration shrinkage measurements and nanoindentation to determine modulus recovery.

Results: After two weeks of remineralization treatment the shrinkage was significantly reduced in all treatment sets when compared to the demineralized control. Nanoindentations placed across the interface indicated improvement for RMGI containing 40% pAsp, and were significantly elevated when lesions were rehydrated with pAsp before being restored with RMGI. In particular the most demineralized outer zone recovered substantially in the elastic modulus, suggesting that functional remineralization has been initiated by pAsp delivery upon rehydration of air-dried demineralized dentin. In contrast, the effectiveness of the RMGI on functional remineralization of dentin was minimal when pAsp was absent.

Conclusion:

The use of these modified cementation techniques could improve current approaches in minimally-invasive dentistry by adding a reliable method to repair dentin damaged by the caries process.

Support: UCSF Catalyst Award "PILP Treatment for the Repair of Dental Caries" and by the Center for Dental, Oral & Craniofacial Tissue & Organ Regeneration (C-DOCTOR) with the support of NIH NIDCR (U24DE026914). Additional support was provided by a 2017 IADR Innov

#56 Title: NCKX4 is critical for extracellular enamel matrix protein removal

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Objectives: Enamel is the hardest and most mineralized tissue in the human body. Maturation ameloblasts are the major cells responsible for removing the organic matrix proteins, while also transporting calcium and phosphate into the enamel space to orchestrate the formation of this tissue. NCKX4, a potassium (K⁺) dependent calcium (Ca²⁺)/sodium (Na⁺) exchanger, is highly expressed in the maturation stage of ameloblasts. Mice with the loss-of-function of NCKX4 (Nckx4^{-/-}) display poorly mineralized enamel and a disorganized maturation ameloblast layer. This goal of this study is to investigate the role of NCKX4 in the removal of the enamel matrix proteins and enamel formation.

Methods: Hemimandibles from Nckx4^{+/+} and Nckx4^{-/-} mice were subjected to microCT analyses to determine the mineral density of craniofacial hard tissues. First molars from Nckx4^{+/+} and Nckx4^{-/-} mice were dissected on postnatal day 5 and 12, and total RNA was extracted to synthesize cDNA libraries, which served as templates for RT-qPCR reactions. Maturation stage of enamel matrix proteins extracted from Nckx4^{+/+} and Nckx4^{-/-} mouse first molars were separated on SDS-PAGE gels followed by Coomassie staining and Western Blotting. Sagittal sections of hemimandibles from Nckx4^{+/+} and Nckx4^{-/-} mice on postnatal day 21-22 were paraffin-embedded and processed for immunohistochemistry staining and RNAscope analyses.

Results: Nckx4^{-/-} mice had significantly reduced enamel mineral density than Nckx4^{+/+} mice. Amelogenins were retained in the maturation stage Nckx4^{-/-} mouse enamel matrix although Klk4 transcripts were upregulated in Nckx4^{-/-} mouse transition and mature ameloblasts compared to that of the wildtype. LAMP1 and Cathepsin L, proteins associated with lysosome activities, were delocalized in the Nckx4^{-/-} maturation ameloblasts, whereas they were distributed in the cytosol close to the apical pole of wildtype maturation ameloblasts.

Conclusion: NCKX4 is involved in the proper removal of enamel matrix proteins from the extracellular matrix through regulating the enzymatic hydrolysis and endocytosis of amelogenins.

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