38TH ANNUAL

# RESEARCH DAY

# SPEAKER ABSTRACTS

Friday, February 24, 2023

School of Dental Medicine

UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

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**Carmem Pfeifer, DDS, PhD,** is a Professor in the Division of Biomaterials and Biomechanics at Oregon Health Sciences University, School of Dentistry.

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**Thomas E. Forman, BS,** is a MD-PhD Candidate; Department of Craniofacial Biology and works in the laboratory of Dr. Katherine Fantauzzo.

*Title: Alternative RNA splicing downstream of PDGFRa signaling in craniofacial development.*

**Natalie Anderson, BS.** is a Doctor of Dental Surgery Candidate (2026) and was a FY22 summer scholar recipient in the laboratory of Dr. Jefferey Stansbury, Department of Craniofacial Biology.

*Title: Design of Robust, Non-Covalently Reinforced Composite Materials*.

**Elise Ambrose, BS,** is a Doctor of Dental Surgery Candidate (2024) and a research volunteer in the laboratory of Dr. Devatha Nair, Department of Craniofacial Biology.

*Title: Light-propelled dental adhesives with enhanced bonding capability*.

**Stanley M. Kanai, PhD,** is a Postdoctoral Fellow, Department of Craniofacial Biology and works in the laboratory of Dr. David Clouthier.

*Title: Lower Jaw Development is Mediated Solely by the q/11 Class of G Proteins.*

Title: **Host and Bacterial Enzyme-Mediated Approaches to Produce Durable Biomaterials: Trends in Polymeric Materials for Dental Applications**

Presenter: **Carmem Pfeifer, DDS, PhD**

Oregon Health & Science University School of Dentistry

Dr. Carmem Pfeifer (DDS, PhD) is Professor in the Division of Biomaterials and Biomechanics at OHSU School of Dentistry. Dr. Pfeifer teaches Dental Materials and serves as an instructor in several Restorative Dentistry pre-clinical disciplines. She has published over 100 research articles in the field of Dental Materials Sciences and Polymer Chemistry and serves as a standing member of the DSR study section for the National Institute of Dental and Craniofacial Research at NIH. She has been recently appointed Associate Editor for the Journal of Dental Research. Dr. Pfeifer's research focuses on the development of innovative polymeric materials for restorative dentistry, and she has received the inventor of the year award from OHSU for the commercial potential of her patented inventions, which have attracted the interest of several potential licensees. She has received over 12 million dollars in funding for her research and career development from the National Institutes of Dental and Craniofacial Research (including the hyper-competitive R35 – Sustaining Outstanding Achievement in Research award), Oregon Medical Research Foundation, National Science Foundation, as well as industry partners.

**ABSTRACT:**

Dental caries continues to be a public health issue, especially more evident in underserved populations throughout the U.S. Unfortunately, especially with an ageing population, hundreds of thousands of resin composite restorations are replaced each year due to recurring decay and fracture. According to a number of cohort studies, the average life-span of this type of restoration is 10 years or less, depending on the caries risk level of the patient and on the complexity of the restorative procedure. Any new material development must depart from the simple restoration of form paradigm, in which the filling is simply inert/biocompatible. This talk will discuss novel antibiofilm structures, based on a targeted approach specifically against dysbiotic bacteria. Biofilm coalescence can be prevented by using GTF inhibitors, in a non-bactericidal approach. On the tooth substrate side, MMP-inhibiting molecules can improve the stability of the collagen in the hybrid layer. This talk will also discuss the importance of testing the materials in a physiologically relevant environment, mimicking the conditions in the mouth in terms of mechanical loading, bacterial challenge, and presence of saliva. Ultimately, the goal of materials development is to achieve durable restorations, capable of adapting to the oral environment and resisting challenges that go beyond mechanical demands. That way, we can prevent the unnecessary loss of additional tooth structure that comes with every re-treatment.

Title: **Alternative RNA splicing downstream of PDGFRa signaling in craniofacial development**

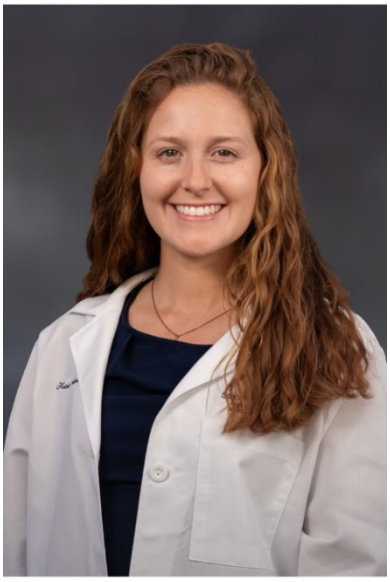
Presenter: **Mr. Thomas Forman, MD-PhD Candidate**

University of Colorado Anschutz Medical Campus, School of Dental Medicine, Department of Craniofacial Biology

Mr. Forman attended the University of Oregon where he obtained his Bachelor of Science in 2017 with honors. While at the University of Oregon, he worked in the laboratory of Dr. Kryn Stankunas investigating the role of receptor tyrosine kinase signaling in cardiovascular development in mice. In 2018, he entered the MD-PhD program at the University of Colorado, and in 2021 joined the lab of Dr. Katherine Fantauzzo in the Department of Craniofacial Biology. In 2022, he was awarded a F31 Fellowship from the National Institute of Dental and Craniofacial Research (NIDCR). His current research as an NIDCR-funded predoctoral fellow focuses on the mechanisms by which receptor tyrosine kinases, specifically platelet-derived growth factor receptor alpha, control gene expression during mammalian craniofacial development.

**ABSTRACT:** Signaling through the platelet-derived growth factor receptor alpha (PDGFRa) plays a critical role in craniofacial development, as mutations in *PDGFRA* are associated with cleft lip/palate in humans and *Pdgfra* mutant mouse models display varying degrees of facial clefting. Phosphatidylinositol 3-kinase (PI3K)/Akt is the primary effector of PDGFRa signaling during skeletal development in the mouse. We previously demonstrated that Akt phosphorylates the RNA-binding protein serine/arginine-rich splicing factor 3 (Srsf3) downstream of PI3K-mediated PDGFRa signaling in mouse embryonic palatal mesenchyme (MEPM) cells, leading to its nuclear translocation. We further showed that ablation of *Srsf3* in the murine neural crest cell lineage results in severe midline facial clefting, due to defects in proliferation and survival of cranial neural crest cells, and over a thousand differential alternative RNA splicing events. We hypothesize that PI3K/Akt-mediated PDGFRa signaling regulates Srsf3 protein and RNA interactions to affect the alternative RNA splicing of transcripts necessary for craniofacial development. Here, we demonstrate via enhanced crosslinking and immunoprecipitation (eCLIP)-seq analysis of MEPM cells that PDGF-AA stimulation leads to preferential binding of Srsf3 to exons. Further, an unbiased motif enrichment analysis of Srsf3 binding sites revealed a loss of binding to canonical Srsf3 CA-rich motifs in stimulated samples, indicating that phoshorylation of Srsf3 may affect the strength of its interaction with RNA. Through the analysis of complementary RNA-seq data, we show that the subset of transcripts that are bound by Srsf3 and undergo alternative splicing upon PDGFRa signaling commonly encode regulators of Wnt signaling, a pathway known to be critical for mammalian craniofacial development. Taken together, these findings provide considerable insight into the mechanisms underlying gene expression regulation during mammalian craniofacial development.

Funded by NIDCR/NIH F31DE032252 (to T.E.F.) and R01DE030864 (to K.A.F.)



Title: **Design of Robust, Non-Covalently Reinforced Composite Materials**

Presenter: **Ms. Natalie Anderson, BS, DDS Candidate (2026)**
University of Colorado Anschutz Medical Campus, School of Dental Medicine

Her research training began in May 2022 prior to starting dental school when she was awarded a position as a Summer Research Scholar. She trained in the Stansbury Biomaterials Laboratory at the University of Colorado School of Dental Medicine. Her research project was to design water tolerant non-covalently reinforced composites with Dr. Stansbury and his senior laboratory manager. The experience and knowledge she gained during this time enabled her to place 1st in the CU School of Dental Medicine Research Competition in Fall 2022. Additionally, her research abstract was recently accepted at the American Association for Dental, Oral, and Craniofacial Research Annual Meeting this March in Portland, Oregon. Ms. Anderson will present her research findings at a scientific session and compete in the Student Competition for Advancing Dental Research and its Application (SCADA). Ms. Anderson is passionate about education and am interested in becoming a Pediatric Dentist with a potential career in academia.

**ABSTRACT:** This study investigates the use of non-covalent interactions within polymer networks to enhance mechanical properties as well as water tolerance or even water-based strengthening of new dental composites.

Novel tetraurethane diacrylate (TUDA) monomers were combined with acrylic acid (AA) and 4-methacryloxyethyl trimellitic anhydride (4-META) at different ratios relative to urethane functional groups. Resin composites were formulated with 0.7 μm silanized filler at an initial but not limiting 60 wt% loading. Filtek Bulk Fill (FBF, 3M) was included as a commercial composite control. The degree of conversion of photopolymerized samples was measured via FT-Near-IR spectroscopy and both dry and water conditioned 3-point bend testing provided flexural strength, modulus, and toughness results.

Isophorone tetraurethane diacrylate (ITUDA) or xylylene tetraurethane diacrylate (XTUDA) was used as the crosslinking comonomer along with 4-META and AA. Ambient photocure conversion was extremely high for the unfilled resins (>90%) while the experimental composites showed a modest reduction (>80%) although still dramatically higher than conversion of the FBF control. In a dry environment, the unfilled resins displayed unusually high flexural strength, which upon exposure to water declined to the level of the wet composite control. However, once filler was introduced in the TUDA formulations, the wet composite strength and modulus increased significantly relative to their corresponding dry properties. Wet urethane composites matched the outstanding flexural strength results achieved with the unfilled dry polymers although the composite toughness either dry or wet was well below that of the unfilled polymers. Composite modulus is directly related to filler content, which was not optimized here. These composite materials were designed to achieve higher conversion and higher mechanical performance than conventional dental composites, which was demonstrated here. This study shows the potential for non-covalently reinforced urethane-based composites that rely on both acid and hydrolytically cleavable anhydride interactions to provide water-strengthened dynamic behavior. Partially funded by NIH/NIDCR R21DE028444


**Light-propelled dental adhesives with enhanced bonding capability**

Presenter: **Ms. Elise Ambrose, DDS Candidate (2024)**

University of Colorado Anschutz Medical Campus, School of Dental Medicine

Ms. Ambrose is a third year DDS student at the University of Colorado School of Dental Medicine who seeks to find novel solutions to traditional challenges within dental materials. As part of the Nair Lab, she has worked with a team of chemical engineers and materials science researchers to test composite restorations using light-propelled adhesives. Having graduated from the Metropolitan State University in 2018 with a major in biology and a minor in chemistry, she has previously won accolades for her work on bed bug microbiomes as part of her undergraduate research work. She is also the recipient of multiple research awards at the University of Colorado School of Dental Medicine and has most recently won a 2023 student travel grant to present her at work at the American Association of Dental, Oral and Craniofacial Research meeting in Oregon. Ms. Ambrose loves animals and works as a keeper assistant at the Denver Zoo when her schedule permits.

**ABSTRACT:** Failure of composite restorations is attributed to a weak hybrid layer, which bonds the adhesive to the tooth structure. By introducing light-responsive nanoadditives (1- 5 wt.%) within conventional adhesives, we propose to drive the controlled, light-activated, diffusion of adhesive resin up to the depth of etching towards strengthening the hybrid layer. Additionally, pendant functionality on the nanoadditives will enhance the resin-dentin interface via secondary bonds.

Light-responsive nanoadditives were synthesized, characterized and incorporated within BisGMA/HEMA adhesives (degree of conversion, 6163 $cm^{-1}$, near-FTIR). Ten human molars were etched (37% phosphoric acid), and adhesives with/without nanoadditives were placed on the prepared tooth surface. Confocal images (Nikon A1R) captured the response to visible light (430-480 nm, 60s, 700 $mW/cm^2$). Flexural strength tests (n = 8, 25 mm × 2 mm × 2 mm, MTS Mini Bionix II) characterized the enhanced strength of adhesives formulations.

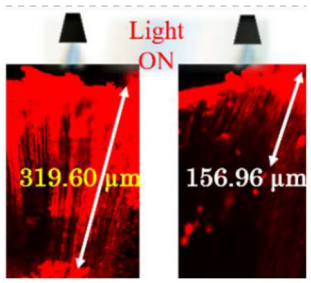



**Fig. 1**. Confocal images show with nanoadditives (left) can propel flow away from the light and infiltrate dentinal tubules up to the depth of etch in comparison to a control (right).

Nanoadditives (GPC: Mw = 12 kDA, Rh = 1.74 nm) within adhesives responds to 430-480 nm light with high degree of conversion (98.6 ± 0.3%). Nanoadditives increased depth and span of penetration within dentinal tubules (**Fig. 1**). Flexural tests with/without nanoadditives show enhanced toughness (Toughness = 5 ± 1 mJ/mm3 vs Toughness = 0.8 ± 0.2 mJ/mm3).

Light-responsive nanoadditives can drive the controlled, light-activated, homogenous diffusion of adhesive resin components within dentinal tubules. Pendant functionality on nanoadditives can enhance the strength of adhesive resins via secondary interactions. Future tests will include characterizing the mechanical strength of the adhesive/tooth via µTBS tests in physiological conditions.

*Funding support: NIH/NIDCR K25DE027418*

Title: **Lower Jaw Development is Mediated Solely by the q/11 Class of G Proteins**

Presenter: **Stanley (Michi) Kanai, PhD**

University of Colorado Anschutz Medical Campus, School of Dental Medicine, Department of Craniofacial Biology

Dr. Kanai graduated from the University of California, San Diego in 2008 with a Bachelor of Science in Neuroscience and Physiology. As an undergraduate he worked as a research assistant in the laboratory of Dr. Geoff Rosenfeld, performing research on eukaryotic gene regulation. In 2010, he transitioned to Washington University in St. Louis School of Medicine and received his PhD in Molecular Cell Biology. He trained in the laboratory of Dr. Kendall Blumer, with his dissertation research on the regulatory mechanisms and pharmacology of G protein-coupled receptor signaling. Following the completion of his doctorate in 2017, Dr. Kanai began his postdoctoral training in the laboratory of Dr. David Clouthier in the Craniofacial Biology department at the University of Colorado Anschutz Medical Campus. His current studies involved mechanisms of craniofacial development and disorders. In 2020, Dr. Kanai was awarded an F32 Fellowship Award from the National Institute of Dental and Craniofacial Research to investigate the role of heterotrimeric G proteins in zebrafish craniofacial development, under the guidance of co-mentors Drs. David Clouthier and James Nichols.

**ABSTRACT:**

In jawed vertebrates, many skeletal elements and connective tissue of the craniofacial complex are derived from cranial neural crest cells (NCC) of the first pharyngeal arch (PA1). NCC identity is established by various ligand-receptor signaling pathways that determine when and where patterning genes are expressed along the dorsal-ventral (D-V) axis of PA1. Endothelin-1 (Edn1) and Endothelin receptor type A (Ednra) represent one of these ligand-receptor pairs and are required to establish the ventral and intermediate identities. Loss of Edn1 or Ednra results in a homeotic transformation of the lower jaw into upper jaw-like structures in zebrafish, chick, mice and humans.

While the patterning genes regulated by Edn1/Ednra are well characterized, signaling mechanisms that link Ednra activity to gene regulation are not fully understood. Ednra is a G protein-coupled receptor that can activate all classes of G proteins – Gq/11, Gi/o, Gs, and G12/13. Based on studies in mice, we and others hypothesized that one or more G protein classes are responsible for establishing the intermediate and ventral identity domains in PA1, with Gq/11 driving intermediate identity (jaw joint precursor), and Gi/o or Gs driving ventral identity (distal jaw precursor).

We tested this hypothesis using genetic and transgenic approaches in Zebrafish. CRISPR/Cas9 was used to generate knockout alleles for the three genes that encode Gq/11 proteins, *gnaq*, *gna11a*, and *gna11b*, and their effects on lower jaw development were analyzed. We found that triple homozygous knockout animals produced lower jaw defects that phenocopy *edn1* knockout animals. We then used an inducible transgene to express constitutively active Gq protein in *edn1* knockout animals, and we found that it rescued the lower jaw phenotype. These results indicate that Gq/11 is necessary and sufficient to establish all Edn1/Ednra-dependent patterning domains in zebrafish. Future work will investigate the Gq/11-dependent signaling mechanisms that regulate gene expression.

Funded by NIDCR/NIH F32DE029406 (S.M.K.) and R01DE029091 (D.E.C.).

38TH ANNUAL

# RESEARCH DAY

## STUDENT ABSTRACTS (DDS AND ISP)

Friday, February 24, 2023

School of Dental Medicine

UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

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## ABSTRACTS

### Students of the Doctor of Dental Surgery and the Advanced Standing International Student Program

- *Prevalence of Dental Anomalies in Patients with Wolf-Hirschhorn Syndrome.*
  Authors: Miller, Tyler M, Pickett K, Flaitz CM, Puranik CP.

- *Assessing bias between instructor and computer-graded student-prepared endodontic accesses.*
  Authors: Geck, Nathaniel, Berni Emilia.

- *Acrylated Hydroxyazobenzene Coating over Resin-Based Sealants Inhibits Streptococcus mutans.*
  Authors: Paoli Francis, Schurr Michael, Puranik CP, Nair Devatha, Kehe Gannon, Kadur Pinky.

- *High Strength, High Toughness 3D Printable Urethane Denture Base Formulations*.
  Authors: Monley, Simon, Kehe Gannon, Stansbury Jeffrey.

- *Silver diamine fluoride: testing an approach to control staining*.
  Authors: Meola, Brittany, Carey Clifton.

- *A Comparison of Hand K- Files, One Shape and ProTaper Next Rotary Systems for Elimination of Enterococcus Faecalis from Root Canal: Microbiological and SEM Evaluation*.
  Authors: Bala Sroa R, Sidhu B, Aggarwal S, Chopra A, Bedi Angelina.

- *Investigating the Role of Gq/11 Proteins in Zebrafish Craniofacial Development*.
  Authors: Garcia, Chloe R, Kanai Stanley., Augustus MaCalia, Sharafeldeen Shujan, Brooks Elliot, Lencer Ezra, Nichols James T, Clouthier David.

Doctor of Dental Surgery and International Student Program Candidates, School of Dental Medicine

Title: **Prevalence of Dental Anomalies in Patients with Wolf-Hirschhorn Syndrome**

Authors: **Miller Tyler M**[1*], Pickett K[2], Flaitz CM[3], Puranik CP[4]

*Presenting author, [1]Doctor of Dental Surgery Program, School of Dental Medicine, [2]Research in Outcomes for Children's Surgery [3]Division of Diagnostic Sciences, School of Dental Medicine, [4]Pediatric Dentistry Residency Program, Children's Hospital Colorado, and School of Dental Medicine. University of Colorado, Aurora, CO, USA.

**Purpose:** The purpose of this retrospective, observational cohort study was to evaluate the prevalence of developmental dental anomalies and pathoses (DDAP) in children with Wolf-Hirschhorn Syndrome **(**WHS).

**Methods:** One million electronic medical-dental record of patients (1-18years) reporting at Children's Hospital Colorado were screened for WHS diagnosis. Twenty-six charts identified with WHS diagnosis were systematically screened by a calibrated examiner for medical and dental information including dental anomalies of shape, number, position, structure, and other developmental anomalies or pathoses. The collected data was descriptively summarized.

**Results:** All the reviewed WHS patients had otorhinolaryngological, speech or behavioral findings while four-fifths of the patients had gastrointestinal, musculoskeletal, central, or peripheral nervous system findings. Two-thirds of the WHS patients had respiratory and genitourinary findings while ophthalmological and cardiovascular findings were documented in about half of the cases. Endocrine and hematological findings were relatively rare. All the WHS patients had positive craniofacial findings including microcephaly and dysmorphic features while two-thirds had maxillary excess. Twelve children with WHS had complete clinical and radiographic dental documentation. Of these, Microdontia (83.3%), pyramidal molars (66.7%), taurodontism (50%), dilacerated roots (33.3%), dens invaginatus (16.7%), pulp stones (16.7%), and root anomalies (16.7%) were the most common shape anomalies. There was a high prevalence of number anomalies (hypodontia:50.0%) while the most common positional anomalies included rotated (25%), ectopic (16.7%), infra-occluded (8.3%), distally displaced (8.3%), or impacted (8.3%) teeth.

**Conclusion:** The study provides a comprehensive review of medical-dental findings in WHS patients to assist pediatric dentists in the detection and management of DDAP.

Title: **Assessing bias between instructor and computer-graded student-prepared endodontic accesses**

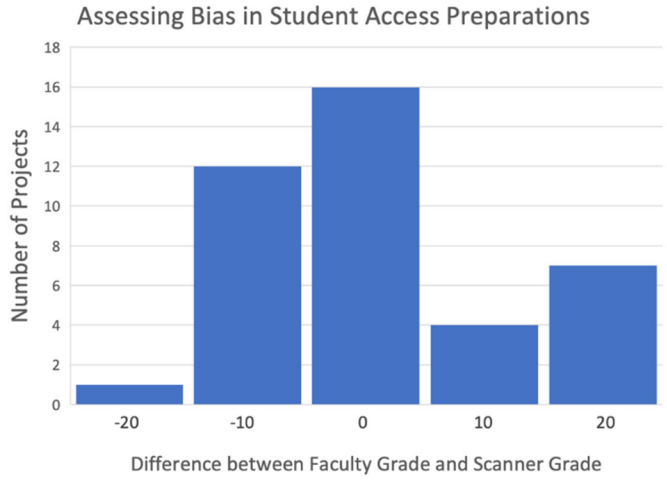
Authors**: Nathaniel Geck* and Emilia Berni**

*Presenting author, Doctor of Dental Surgery Program, School of Dental Medicine

**Purpose:** This project sought to evaluate the difference between subjective faculty-graded preclinical lab work and objective technology-graded preclinical lab work in dental school. With the increased use of handheld scanners, the traditional subjective process of grading dental students' lab projects can potentially be changed to an objective one, free of any form of bias. Eliminating potential biases is especially important in academic settings, where students are constantly being evaluated.

**Methods:** Final projects from previous endodontic courses were collected and scanned using Trios4 scanning technology. Using this scan data and a CloudCompare program, the projects were compared to determine the percent difference between the student's and the ideal access. After an objective similarity score was obtained for each project, those scores were statistically compared using a Kolmogorov-Smirnov test to determine if there was a significant statistical difference between the two grading styles.

**Results:** A Kolmogorov-Smirnov test was used to compare the difference between the two grading styles to a function of zero. It was determined that there was statistical significance between the subjective and objective grading methods. Comparing the results with a function representing no change shows the discrepancy between the two grading methods. The objectively graded preps tended to score higher than the subjectively graded projects. Additionally, the test showed that most preps scored similarly between the two grading systems




**Conclusions:** Our data provided evidence that there is a statistical difference between a subjective and objective grading style when evaluating endodontic access preps. We hypothesize, based on the subjective grading notes, that most of the projects that received a lower score were due to extraneous factors related to the preparation process. This shows a limitation in the scanner's ability, but also introduces bias in the instructor grading process.

Title: **Acrylated Hydroxyazobenzene Coating over Resin-Based Sealants Inhibits *Streptococcus mutans***

Authors: **Paoli Francis***, Schurr Michael, Puranik CP, Nair Devatha, Kehe Gannon, and Kadur Pinky.

*Presenting author, Doctor of Dental Surgery Program, School of Dental Medicine

**Purpose:** This *in-vitro* study investigated the inhibitory potential of an acrylated hydroxyazobenzene (AHA) coating over resin-based pit-and-fissure sealants on *Streptococcus mutans* (*Sm*) growth.

**Methods:** Commercially available sealant was applied on occlusal surfaces of extracted human molars (n=12) and cured for 40seconds as per manufacturer's instructions. AHA coating 4.2±0.4µl was placed over the sealants and cured for 40seconds. AHA-coated (test) and uncoated molars (control) were subdivided into 2 groups with or without

6months equivalent toothbrushing. All samples were washed with 70% ethyl alcohol for 15minutes, followed by 5minutes of UV-irradiation. Substrates (sealed molars±AHA) were incubated in phosphate-buffered saline containing 1% Penicillin-Streptomycin overnight and then washed. *Sm* ($10^{!}$) were seeded on substrates and cultured for 24hours at 37℃ and 5% $CO_2$ in Brain Heart Infusion Agar (BHI) with 1% sucrose. Media was replenished after 24hours. At 48hours, substrates and surrounding media were sonicated, serially diluted, and seeded onto BHI plates. *Sm* on substrates and surrounding media were quantified using colony forming units.

**Results:** Dilution from AHA-coated molars and surrounding media did not demonstrate growth of a single *Sm* colony on BHI plates. Similarly, 6months toothbrushing did not impact the AHA-mediated *Sm* inhibition. In contrast, dilutions from uncoated molars and surrounding media, regardless of toothbrushing, demonstrated proliferation of abundant *Sm* colonies ($10^{"}$-$10^{7}$).

**Conclusion:** AHA-coating over sealants inhibited cariogenic *Sm* growth, *in vitro*, while demonstrating a zone of inhibition around the substrate (envelope effect). The AHAmediated inhibition of *Sm* was intact even after 6months equivalent toothbrushing.

Funding source: Gates Grubstake 63404365 (C.P., D.N., M.S.)

Title: **High Strength, High Toughness 3D Printable Urethane Denture Base Formulations**

Authors: **Simon Monley***, Gannon Kehe, Jeffrey Stansbury

*Presenting author, Doctor of Dental Surgery Program, School of Dental Medicine

**Objectives:** Interest in 3D printed denture technology has increased significantly, but current 3D printed denture base resins share the same limitations as PMMA denture base resins of limited flexibility, toughness, and minimum thickness. We formulated low viscosity, all-urethane resins to maximize the non-covalent interactions between urethane groups known to enhance polymer strength and toughness.

**Methods:** Resins containing 1:1 or 2:1 molar ratio of monourethane dimethacrylate (MUDMA) and diurethane dimethacrylate (UDMA) were formulated. Photopolymer 3D printing was simulated through ambient photocuring and photo/thermal post-cure. Degree of conversion was obtained using FTIR spectroscopy. Samples from wet and dry storage conditions were subjected to 3-point bending with flexural strength, modulus, and toughness recorded.

**Results:** Viscosities for 1:1 (402.1mPas) and 2:1 (204.8mPas) MUDMA/UDMA resins were within vat-printable range without addition of urethane-diluting comonomers. Wet flexural strength of 1:1 (157.5±8.5MPa) and 2:1 (155.3±5.2MPa) MUDMA/UDMA polymers was significantly greater ($P\sim0$) than heat-cured PMMA (93.3±3.5MPa). Elastic modulus of 1:1 (2.8±0.1GPa) and 2:1 (3.1±0.1GPa) MUDMA/UDMA formulations were below ($P<0.05$) that of PMMA (3.6±0.4GPa), which may facilitate patient comfort. The urethane-based base polymers also demonstrated higher strain to failure than brittle PMMA. With modest variation of these formulations, tough photopolymers with similar modulus (~3 GPa), but wet flexural strength of >200 MPa, were obtained.

**Conclusions:** The urethane-based denture base resins developed in this study produce polymers of substantially increased strength and toughness relative to PMMA. These resins would allow for fabrication of thinner, more deformation-tolerant printed dentures, potentially leading to fewer pre-prosthetic surgeries and broken dentures. These versatile resins demonstrate properties suitable for vat 3D printers, as well as for new inkjet 3D printers just coming to market.

Funding source: NIH/NIDCR R21 DE028444 (J.W.S.)

Title: **Silver diamine fluoride: testing an approach to control staining**

Authors: **Brittany Meola*,** Clifton Carey

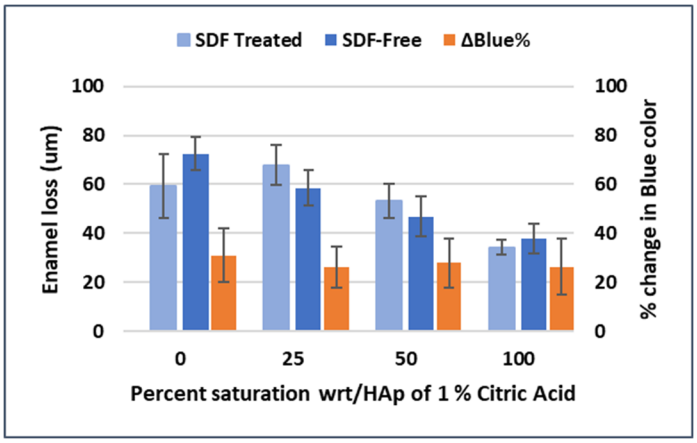
*Presenting author, Doctor of Dental Surgery Program, School of Dental Medicine

**Introduction:** Silver diamine fluoride (SDF) is popularly used to halt caries progression by reducing the bacterial load and depositing fluoride in a lesion. One side effect is that the SDF generates a persistent black stain on the lesion over time. Our purpose was to evaluate a method to reduce SDF staining without demineralizing the tooth. Our hypothesis was that citric acid (CA) solution saturated with respect to hydroxyapatite (wrt/HAp) will significantly reduce SDF stain.

**Methods:** Forty-eight caries-free enamel samples (2x2x1)mm were cut from 1st and 2nd human molars, sanded at 400grit to remove surface fluoride and divided into 2 groups SDF-treated and nonSDF-treated. The SDF-treated samples were etched, treated with SDF for 2min and photographed for staining over 6d. Subgroups (n=6 each) of SDF and nonSDF samples were submitted to 1% pH 3.6 CA solutions of varying saturation (0, 25, 50, 100)% wrt/HAp for 4h. Surfaces of SDF-treated samples were rephotographed. All samples were cross-sectioned, and erosion depth was determined following the light microscope method in ISO28399. Change in stain of SDF samples was estimated from the difference (before/after CA challenge) in blue hue (ΔBlue%) using Image-J.

**Results:** 2-way ANOVA found that erosion of SDF and nonSDF samples were not significantly different at each CA/HAp saturation level ($p=0.8865$); however, there were significant differences in erosion between HAp saturation levels ($p<0.0001$). A significant interaction between SDF treatment and erosion ($p=0.0042$) was observed. The intensity of the stain was reduced by 28%, however ANOVA found no significant differences in the ΔBlue% between the %saturation groups ($p=0.8524$).

**Conclusion:** SDF offered no additional protection from CA erosion; %Saturation wrt/HAp of CA significantly reduced erosion. Our hypothesis is supported where the SDF induced stain was significantly reduced with minimal erosion at 100% saturation wrt/HAp.

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Future work:

- Use dentin samples (not enamel)
- Use a different acid (phosphoric)
- Saturate acid solution with powdered enamel or dentin
- Refine ΔBlue% measurement – maybe grey scale

Funding source: School of Dental Medicine Summer Scholar Program

Title: **A Comparison of Hand K- Files, One Shape and ProTaper Next Rotary Systems for Elimination of *Enterococcus Faecalis* from Root Canal: Microbiological and SEM Evaluation**

Author: **Angelina Bedi**, ISP Candidate

Renu Bala Sroa[1], Baljeet Sidhu[1], Sangeeta Aggarwal[1], Aakanksha Chopra[1], Angelina Bedi[1]

[1]Department of Conservative Dentistry and Endodontics, Punjab Government Dental College & Hospital, Amritsar, India

**Citation:** Bala Sroa, R., Sidhu, B., Aggarwal, S., Chopra, A., & Bedi, A. (2018). A Comparison of One Shape and ProTaper Next Rotary Systems for Elimination of Enterococcus Faecalis from Root Canal: Microbiological and SEM Evaluation. *American Journal of Internal Medicine, 6*(5), 126. https://doi.org/10.11648/j.ajim.20180605.16

**Purpose**- Incomplete removal of micro- organisms from infected root canals is a common cause of failed endodontic treatment. The difficulty in eradication of *Enterococcus faecalis* from root canals plays an essential role in pathogenesis of persistent pulpal and periradicular infections. The aim of the present study was to compare the reduction of Enterococcus faecalis in root canals by mechanical instrumentation using two rotary systems (One Shape and ProTaper Next) and Hand K- file instrumentation by using microbiological and Scanning Electron Microscopy (SEM) evaluation.

**Methods**- Fifty-one freshly extracted mandibular premolars with a single root were collected. After pre- instrumentation sampling, they were divided into three groups, Group A, Group B and Group C in which biomechanical preparation was done using Hand K- File, OneShape and Protaper Next respectively. Reduction in pre- instrumentation and post- instrumentation values of *Enterococcus faecalis* were analyzed using microbiological and SEM evaluation.

**Results**- Statistical analysis by paired 't' test and p value showed that there was highly statistical significant difference in CFU count reduction between the pre-instrumentation and post-instrumentation values in all the groups ($p<0.001$). The results of the present study revealed, that the mean percentage reduction of E. faecalis after instrumentation in Group A (Hand K-File) was 95.51%, Group B (OneShape Apical) 97.74% and Group C (ProTaper Next) 98.13%. Statistical analysis for the SEM by Kruskal-Wallis Test and Mann-Whitney Test showed that at 1mm and 3mm level, Group A (Hand K-File) scored significantly higher value followed by Group B (OneShape Apical) and Group C (ProTaper Next). The mean scores for bacteria were least for ProTaper Next followed by OneShape and Hand K-File at both 1mm and 3mm levels.

**Conclusion**- The most effective instrumentation technique in eliminating *Enterococcus faecalis* from the root canal was ProTaper Next system in comparison to OneShape Apical and Hand K-File.

Title: **Investigating the Role of Gq/11 Proteins in Zebrafish Craniofacial Development**

Authors: **Chloe R. Garcia*[1]**, Stanley M. Kanai[1], MaCalia R. Augustus[1], Shujan A. Sharafeldeen[1], Elliot P. Brooks[1], Ezra S. Lencer[2], James T. Nichols[1], and David E. Clouthier[1]

*Presenting author, Doctor of Dental Surgery Program, School of Dental Medicine

[1]Department of Craniofacial Biology, School of Dental Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO USA. [2]Department of Biology, Lafayette College, Easton, PA USA

**Purpose**: Endothelin-1 (Edn1) and Endothelin receptor type A (Ednra) are crucial for craniofacial development in all jawed vertebrates. In humans, dysregulation of Edn1-Ednra signaling is associated with craniofacial birth defects. The Edn1-Ednra signaling pathway drives lower jaw development by regulating the expression of patterning genes in the first pharyngeal arch (PA1). Genes regulated by Edn1-Ednra have been characterized, but the signaling pathways that connect receptor activity to gene regulation are not fully understood. Multiple classes of heterotrimeric G proteins mediate the Ednra signaling pathway, and it remains unclear which G protein classes and their downstream signaling effectors regulate gene expression. We aim to identify the G proteins that mediate Edn1-Ednra patterning mechanisms to improve our understanding of human craniofacial development and disorders.

**Methods**: We used the zebrafish model to examine the role of the q/11 class of heterotrimeric G proteins (Gq/11) in craniofacial development. We used CRISPR/Cas9 to create knockout alleles for the genes that encode Gq/11 proteins, g*naq*, *gna11a*, and *gna11b,* and then examined their effects on lower jaw development. Knockout animals were identified with genotyping assays and stained with Alizarin Red and Alcian Blue to visualize the facial skeleton.

**Results**: Triple homozygous knockout animals failed to form skeletal structures of the lower jaw. Further, Gq/11 triple KO animals phenocopied *edn1* knockout animals. We also observed that different combinations of knockout alleles produced a spectrum of phenotypes ranging from mild to severe. Severe phenotypes correlated with loss of *gna11b* and *gna11a*, whereas milder phenotypes correlated with loss of *gnaq*.

**Conclusion**: Our results indicate that Gq/11 is the sole mediator of Edn1-Ednra's patterning mechanism for lower jaw development. We can now begin to explore the signaling pathways that connect Ednra activation to patterning gene expression. Elucidating these mechanisms will help us better understand, diagnose, and treat human craniofacial disorders.

Funding source: School of Dental Medicine Summer Scholar Program

Title: **A Paw-sitive Dentist Appointment: The Impact of Providing Therapy Dogs in Dental Appointments on the Experience of Dental Anxiety in Veteran Patients**

Author: Lexi Dunnells, BS* Preceptor: Dr. Sheila Stille

*Presenting author, [1]Doctor of Dental Surgery Program, School of Dental Medicine, and Master of Public Health Candidate

**Purpose:** Dental anxiety is a barrier to oral healthcare utilization. Existing research suggests that U.S. military veterans may be more likely to experience dental anxiety and therefore more likely to avoid dental treatment. Providing therapy dogs chairside during a dental appointment could be an effective intervention to minimize dental anxiety and increase oral healthcare utilization among veteran patients. The purpose of this study is to assess the impact of providing therapy dogs during dental appointments on the experience of dental anxiety in veteran patients at the University of Colorado School of Dental Medicine.

**Methods:** In a quasi-experimental study from September to November 2022, twenty veteran patients scheduled in the student clinics were identified. Ten patients had a therapy dog present during their appointment and ten did not. Pre and post measurements of anxiety (pulse, blood pressure, Anxiety Scale surveys) were completed. A data analysis compared measures pre to post and compared measures experimental to control, using appropriate descriptive and paired t-tests with SPSS software.

**Results:** Reduction in pulse was highly significant post-intervention (p-value < 0.01). Experimental patients with a therapy dog present during the appointment had a lower mean pulse compared to control patients without a dog present (64.50 and 76.00 respectively). Experimental patients had a lower mean systolic blood pressure compared to control patients (131.75 and 136.75 respectively).

**Conclusions:** Providing therapy dogs in dental appointments appears to result in a significant reduction in pulse, a measure of anxiety.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

38TH ANNUAL

# RESEARCH DAY

## GRADUATE STUDENT ABSTRACTS

Friday, February 24, 2023

School of Dental Medicine

UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

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**ABSTRACTS**

**Graduate Students within laboratories of the School of Dental Medicine**

- *Title: Investigation of the antibacterial and regenerative properties of a novel AHA dental coating for the treatment of deep caries*.
  *Authors:* **Sarah Asby***, Erin Binne, Gannon Kehe, Pinky Kadur, Devika Dharmala, Michael Schurr, Chaitanya Puranik, Devatha P. Nair

  **Presenting author is a graduate student in the Nair Lab.*

- *Title: Variable paralog expression underlies incomplete penetrance*.
  *Authors*: **Abigail Mumme-Monheit***, Juliana Sucharov, James T. Nichols

  **Presenting author is a graduate student in the Nichols Lab.*

- *Title: Nanogels for Oral Drug Delivery*.
  *Authors*: **Dixa Gautam***, Humberto Escobedo, Dr. Devatha Nair

  **Presenting author is a graduate student in Bioengineering and Senior Lab professional in the Nair Lab.*

Category: Graduate Students in labs at the School of Dental Medicine

Title: **Investigation of the antibacterial and regenerative properties of a novel AHA dental coating for the treatment of deep caries**

Authors: **Sarah Asby**, Erin Binne, Gannon Kehe, Pinky Kadur, Devika Dharmala, Michael Schurr, Chaitanya Puranik, Devatha P. Nair

**Objective/Goals:**
Our objective is to investigate the antibacterial, regenerative, and wound healing properties of a novel AHA dental coating for the prevention and treatment of deep caries (cavities). Further, we aim to investigate and compare these properties through *in vivo* murine models and assessment on human saliva samples and human gingival fibroblasts.

**Methods:**
*In vitro* antibacterial studies were performed by collecting and culturing human salivary bacteria with AHA substrates and quantifying survival of cariogenic Sm (*S. mutans*). *In vivo*, C57BL/6 mice were treated with AHA composite fillings, infected with Sm clinical isolates, and fed a high sucrose diet with cavity formation assessment after 6 weeks. To evaluate regeneration, mice were similarly given composite with AHA or MTA (n=10) upon pulpal exposure for 2, 4, and 8 weeks, with regeneration quantified by microCT and histological analysis. *In vitro*, AHA substrates were cultured with MC3T3-E1 pre-osteoblast cells and human gingival fibroblasts to assess cell migration using a scratch/wound assay.

**Results/Anticipated Results:**
*In vivo* studies have shown the reduction of cavity formation in mice treated with AHA as well as dentin regeneration upon pulpal exposure using microCT and histological image analysis. Coinciding with these findings, AHA substrates eradicated cariogenic Sm in human saliva samples and single species cultures *in vitro.* Further, preliminary results have shown expedited cell migration of human gingival fibroblasts. We anticipate similar migration as well as increased mineralization and ALP production by human pulpal stem cells from clinical samples when cultured with AHA substrates, suggesting osteogenesis. Further, we anticipate increased odontoblast migration, mineralization, and ALP production upon additional analysis of *in vivo* tissue samples.

**Discussion/Significance of Impact:**
This work will elucidate the antibacterial and regenerative properties of AHA dental coatings. These results further support the translation of AHA coatings into the clinic as a novel therapeutic for the prevention and treatment of dental decay.

Funding sources: NIH/NIDCR K25DE027418-05 (D.P.N.); NCATS CTSA TL1 TR002533-05 (R.S.) and Gates Grubstake 63404365 (C.P.; D.N.; M.S.)

Title: **Variable paralog expression underlies incomplete penetrance**

Authors: **Abigail Mumme-Monheit**, Juliana Sucharov, James T. Nichols

Incomplete penetrance, in which some individuals with a given mutation display a phenotype, and others do not, is present in many genetic diseases. However, the mechanisms of incomplete penetrance remain elusive. Using a zebrafish model of the human craniofacial disease *MEF2C* Haploinsufficiency Syndrome, we have developed a system to study heritable incomplete penetrance. Zebrafish *mef2ca* mutants show a range of incompletely penetrant phenotypes, and after selective breeding, the penetrance of these phenotypes can be driven up or down. There are six zebrafish *mef2* paralogs that arose from whole genome duplications (WGDs), which share highly conserved amino acid sequences. However, *mef2ca* is the only paralog known to have craniofacial functions. We discovered that *mef2* paralogs are more highly expressed in the low-penetrance strain compared to the high-penetrance strain, and that there is standing variation in paralog expression in unselected wild types. These findings indicate that heritable variation in paralog expression underlies heritable incomplete penetrance. Therefore, I hypothesize that epigenetic variation regulating compensatory paralog expression modulates heritable incomplete penetrance. To better understand what genetic and epigenetic factors underlie paralog expression variation, and the resulting incomplete penetrance, I will capitalize on unique strengths of our zebrafish system. First, I will use single cell RNA sequencing (scRNA-seq) and assays for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq) to identify chromatin and gene expression patterns that segregate with incomplete penetrance over multiple generations. Second, I will use CRISPR knock-ins to functionally characterize whether *mef2* paralogs can compensate for the loss of *mef2ca.* These gene swapping experiments will test the hypothesis that *mef2* paralogs can rescue *mef2ca* mutants when their spatiotemporal regulation mimics *mef2ca.* These findings motivate a model in which retention of vestigial, ancestral expression and functions of gene duplicates underlies incomplete penetrance.

Funding source: R01DE029193-02 NIDCR (J.T.N.)

Title: **Nanogels for Oral Drug Delivery**

Authors: **Dixa Gautam**, Humberto Escobedo, Dr. Devatha Nair

**Purpose/Aim:** The aim of this study is to synthesize water dispersible nanogels (NG) for drug encapsulation and localized delivery within the oral cavity.

**Materials and methods:** A one-pot, solution polymerization reaction was used to synthesize one amphiphilic (NG1) and one hydrophilic nanogel (NG2). Hydrophilic monomers, 2-hydroxyethyl acrylate, polyethylene glycol methacrylate (Mn = 500), and polyethylene glycol dimethacrylate (Mn= 750) (25-50 mol.%) and hydrophobic monomer stearyl acrylate (25-50 mol.%) were used in this study. The reaction mixture along with 2, 2'–azobis (2–methylpropionitrile) at 1 wt% as a thermal initiator was maintained at 80 °C in methyl ethyl ketone (MEK) until the double bond conversion reached 70% (FTIR, acrylate peak at 815 $cm^{-1}$). NG were then conjugated with a cell-penetrating peptide (CPP) to facilitate transport across the epithelial layers. NG was characterized using GPC and ZetaSizer™, and tested for cytocompatibility. Small molecule drug mimics (MW< 300) were incorporated within NG and release kinetics was quantified for over 7 days.

**Results:** The combination of hydrophilic and hydrophobic monomers yielded NG with molecular weights from 50-170 kDa and hydrodynamic radii between 10-50 nm.

| | Encapsulation Efficiency | Loading Capacity |
|---|---|---|
| NG1 | 97.0% ± 1.3% | 10.5% ± 1.9% |
| NG2 | 83.2% ± 5.0% | 12.1% ± 1.7% |

NG curbed the burst release of the model small molecules in hydrophilic solutions and maintained sustained release for up 5 days. MTT assay demonstrated 90.1 ± 12.2 % cell viability for the nanogels up to 1000µg when compared to a positive control.

**Conclusions:** The small size and inherent flexibility of NG make them ideal for drug delivery within the oral cavity. The ability to control the encapsulation and drug release kinetics from water soluble NG is the first step towards the controlled, targeted *in-situ* drug release of oral therapeutics.

Funding source: NIH/NIDCR K25DE027418-05 (D.P.N.) and NIH/NIDCR R21AI154360-02 (M.S.)

# 38TH ANNUAL
# RESEARCH DAY

## LAB STAFF PROFESSIONALS ABSTRACTS

Friday, February 24, 2023

School of Dental Medicine
UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

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**ABSTRACTS**

**Laboratory Staff Professionals in the School of Dental Medicine**

- *Title: Self-strengthening Random Copolymers as Additives in Dental Resins*.
  *Authors*: **Kehe Gannon***, Gautam Dixa, Stansbury Jeffrey, Nair Devatha.

  **Presenting author is a Senior Lab professional in the Nair Lab.*

- *Title: alx gene family members act to maintain mesenchymal identity in frontonasal neural crest cells*.
  *Authors*: **Murry Matthew***, Mitchell Jennyfer, Jaime Julio, Bailon-Zambrano Raisa, Nichols James T.

  **Presenting author is a Research Lab professional in the Nichols Lab*.

- *Title: UVB Radiation Affects Paraxial Mesoderm Derived Cell Populations that Give Rise to the Dorsal Fin in Zebrafish*.
  *Authors*: **Jaime Julio*,** Rice M, Bailon-Zambrano Raisa, Gagnon J, Nichols James T.

  **Presenting author is an undergraduate student research assistant in the Nichols Lab.*
  Mr. Jaime is a recipient of the prestigious Maximizing Access to Research Careers (MARC) Undergraduate Student Training in Academic Research (U*STAR) Award.

- *Title:Study of Multi-Urethane Multi-(Meth)acrylates as Candidate High-Performance Photopolymers*.
  *Authors*: **Salazar Austyn*,** Stansbury Jeffrey.

  **Presenting author is a Senior Lab professional in the Stansbury Lab*.

- *The her9 gene is not required for osteoblast maturation*.
  *Authors*: **Dolby Colette***, Nichols James T.

  **Presenting author is a Research Lab professional in the Nichols Lab*.

Laboratory Staff Professionals in the School of Dental Medicine

Title: **Self-strengthening Random Copolymers as Additives in Dental Resins**

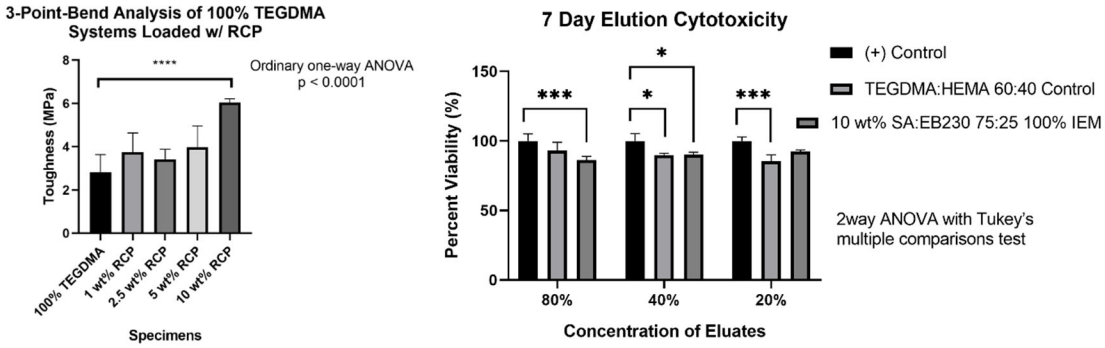
Authors: **Gannon Kehe***, Dixa Gautam, Jeffrey Stansbury, Devatha Nair

*Presenting author is a Senior Lab professional in the Nair Lab, Department of Craniofacial Biology

**Purpose:** Even under near-ideal conditions, photopolymerization of dental adhesive resins leads to inhomogeneous, brittle networks and replacing failed restorations accounts for over 50% of all dental restorative work. The purpose of this study is to synthesize and characterize random copolymers (RCPs) additives within conventional dental adhesives that can drive side-chain crystallization during photopolymerization to generate self-strengthening, tough adhesive networks.

**Methods:** RCPs were synthesized by dissolving Stearyl acrylate and long-chain aliphatic diacrylates at 75:25 molar in ethanol (4X). With 5 wt% mercaptoethanol as the chain transfer agent,1 wt% AIBN was added to initiate the solution polymerization at 85 ℃. Double-bond conversion was monitored via Fourier Transform Infrared Spectroscopy (FTIR) and the reaction was quenched in MilliQ water after 45 minutes. The residual acrylates were endcapped with mercaptoethanol and subsequently reacted with 2-isocyantoethyl methacrylate. The methacrylated-RCPs was added to 100% triethylene glycol dimethacrylate (TEGDMA) and either photopolymerized using dental lamps or 3D-printed in a DLP 3D printer and subsequently characterized using mechanical tests. Using direct contact and elution assays with L929 Mouse Fibroblasts and Human Gingival Fibroblasts, the cytocompatibility of the networks were studied.

Results:




By incorporating RCPs between 1-10 wt% within TEGDMA, a statistically significant increase in toughness is observed. The RCP+TEGDMA networks were cytocompatible when tested with L929 Mouse Fibroblasts and Human Gingival Fibroblast Cells.

**Conclusion:** Structurally weak polymers with low chemical resistance can be reinforced by introducing long-range order - or crystallinity - within the networks. The RCPs drove side-chain crystallization during photopolymerization, as seen in the enhanced toughness observed in the RCP+TEGDMA networks. Future work will focus on the long-term performance of the RCP networks under relevant physiological conditions encountered in the oral cavity.

Funding source: School of Dental Medicine – Nair Lab

Title: ***alx* gene family members act to maintain mesenchymal identity in frontonasal neural crest cells**

Authors: **Matthew Murry***, Jennyfer Mitchell, Julio Jaime, Raisa Bailon-Zambrano, James T. Nichols

*Presenting author is a Research Lab professional in the Nichols Lab, Department of Craniofacial Biology

Craniofacial development requires precise spatiotemporal control over skeletal cell differentiation. A large body of evidence indicates that the *alx* transcription factor encoding gene family is important for craniofacial development in vertebrates ranging from mammals to birds to fish. Previously published studies from the Nichols Laboratory and others have shown expression of *alx* transcription factors to be enriched in the frontonasal population of neural crest cells (fnNCCs). This work also demonstrated that chondrocyte differentiation is precocious in *alx3* mutant fish, chondrocytes develop early. Here, we found that zebrafish a*lx1,* a*lx3*, and a*lx4a* are expressed in a partially overlapping, nested fashion in fnNCCs, resulting in a gradient of *alx* gene family expression. We used lineage tracing to learn that cells in the region with the highest concentration of *alx* genes remain in position and do not produce larval skeletal elements. These findings motivate the hypothesis that high concentrations of *alx* genes function as a “differentiation brake” and inhibit mesenchymal fnNCCs from differentiating into skeletal cells. In support, we find ectopic cartilage develops in this region when alx3 and alx4 are disabled. Further, we find ectopic bone in this region in *alx3* mutant adults. These data motivate a model wherein *alx* expression acts in a dose-dependent manner, and that the highest concentration of *alx* genes represses differentiation of frontonasal mesenchyme into skeletal cells so they are maintained for later development.

Funding source: NIH/NIDCR R01DE029193-02 (J.T.N.)

Title: **UVB Radiation Affects Paraxial Mesoderm Derived Cell Populations that Give Rise to the Dorsal Fin in Zebrafish**

Authors: **Julio Jaime**, Marlen Rice, Raisa Bailon-Zambrano, James Gagnon, James T. Nichols

*Presenting author is a Student Research Assistant in the Nichols Lab, Department of Craniofacial Biology

**Final Abstract:** The effects of ultraviolet radiation (UVR) have been heavily studied in the early development of vertebrates as a means to understand DNA damage in association with various surface level cancers. Previous collected data has been limited to the early embryonic and larval stages of zebrafish. Here we follow early larval ultraviolet-B (UVB) irradiated zebrafish into adulthood through varying exposure treatments to show abnormal dorsal fin development. Furthermore, we observe an increase in the number of zebrafish with reduced or absent dorsal fin appendages as UVB exposure increases. While current research has shown UVR affects surface level ectoderm (skin) and some mesoderm (blood), this study aims to highlight the effects UVB has in skeletogenic paraxial mesoderm derived cells.

**Purpose:** This experiment is being conducted to gain a deeper understanding of the effects of UVR on the development of vertebrates and to extend the previously limited data that has been collected only on the early embryonic and larval stages of zebrafish.

**Methods:** Here we follow early larval ultraviolet-B (UVB) irradiated zebrafish into adulthood at varying exposure treatments to show abnormal dorsal fin development.

**Results:** We observe an increase in the number of zebrafish with reduced or absent dorsal fin appendages as UVB exposure increases.

**Conclusions:** UVB irradiation affects paraxial mesoderm progenitors which result in reduced or absent dorsal fin appendage.

Funding source: NIH/NIDCR R01DE029193-02 (J.T.N.) and NIH/NIGMS MARCU-STAR T34GM096958-10 (R.A.)

Title: **Study of Multi-Urethane Multi-(Meth)acrylates as Candidate High-Performance Photopolymers**

Authors: **Austyn Salazar**, Jeff Stansbury

*Presenting author is a Senior Lab professional in the Stansbury Lab, Department of Craniofacial Biology

**Objective:** This study investigates the use of multi-urethane multi-methacrylates as comonomers to enhance mechanical properties through the use non-covalent interactions.

**Methods:** Different amino alcohols were used in an isocyanate free urethane synthesis to yield core structures that provide for monomers with a variety of urethane and (meth)acrylate ratios. Multi-urethane multi-(meth) acrylates (MU(M)A) were combined with (meth)acrylic acid ((M)AA) comonomer at varied ratios between urethane and acid groups with 2,2-dimethoxy-2-phenylacetophenone used as photoinitator. Polymer samples were prepared using UV irradiation (365nm; 100mW/cm$^2$) and photo/thermal post-curing as a screen for potential 3D printable formulations. Samples were tested under 3-point bending to obtain flexural strength, modulus, and toughness.

**Results:** MU(M)As were designed to simultaneously push limits of mechanical properties and toughness of photopolymers via non-covalent interactions that reinforce covalently crosslinked polymer networks. All MU(M)A resins were shown to attain high flexural strength (>200 MPa) and modulus (>4 GPa) in combination with MAA or AA. PUTriMA + AA (2:1) as well as OUTMA + AA (2:1) were shown to achieve such properties solely through ambient photocuring without post-cure processing. These formulations achieve high strength but are non-brittle with unusually high toughness values. Utilizing non-covalent interactions allows strong yet flexible polymers; however, the interactions are not always straightforward. PUTriMA/AA reaches a flexural strength twice that of PUTriMA/MAA showing how sensitive these MU(M)As can be to comonomer changes. Furthermore, a mixture of AA/MAA attain values as high as 300 MPa which highlights the importance of the non-covalent interactions.

| Urethane Monomer | Acidic Monomer | Ratio (Acid:Urethane) | Cure | Flexural Strength (MPa) | Modulus (GPa) | Toughness (MPa) |
|---|---|---|---|---|---|---|
| PUTriMA | AA | 2:1 | Photo only | 247.9±12.1 | 5.49±0.17 | 19.3±5.3 |
| | | | Thermal | 277.0±20.1 | 5.96±0.46 | 14.0±5.1 |
| PUTriMA | AA/MAA (1:1) | 2:1 | Thermal | *267.8±33.6 | 6.36±0.25 | 8.2±3.0 |
| PUTriMA | MAA | 2:1 | Thermal | 123.8±45.0 | 5.80±0.24 | 1.6±1.2 |
| TetUTriMA | AA | 1:1 | Thermal | 254.7±8.7 | 5.20±0.35 | 13.8±4.3 |
| TetUTriMA | MAA | 1:1 | Thermal | 196.1±39.6 | 5.32±0.13 | 5.2±2.8 |
| TriUDMA | AA | 1:1 | Thermal | 253.6±22.9 | 5.68±0.19 | 9.9±4.5 |
| TriUDMA | MAA | 1:1 | Thermal | 226.9±43.4 | 6.09±0.36 | 5.56±2.48 |
| TriUDMA | AA | 2:1 | Thermal | *271.5±12.0 | 5.03±0.5 | 11.2±2.5 |

*Formulation attained value above 300 MPa flexural strength

**Conclusion:** These MU(M)As were designed to obtain high performance in mechanical properties by use of non-covalent interactions. It has been demonstrated that mechanical properties of >200 MPa flexural strength, >4 GPa modulus, and >10 MPa toughness can be obtained and even pushed further to >250 MPa in flexural strength and >5 GPa in modulus.

Funding source: NIH/NIDCR R21DE028444 (J.W.S.)

Title: **The *her9* gene is not required for osteoblast maturation**

Authors: **Colette Dolby***, James T. Nichols

*Presenting author is a Research Lab professional in the Nichols Lab, Department of Craniofacial Biology

Bones are made by osteoblasts which secrete mineralized matrix. Most of the bones in the head are derived from neural crest cells. Many of the transcription factors encoding genes that control neural crest cell differentiation into osteoblasts are known. However, the subsequent steps of osteoblast maturation are less well understood. In zebrafish, the transcription factor encoding gene *her9* is highly expressed in neural crest cells in 24hr embryos, leading us to believe it has a role in craniofacial development. Using CRISP/Cas9 mutagenesis we generated *her9 mutants*, which resulted in delay or absent bone mineralization. Surprisingly, neural crest cells differentiate into osteoblasts at the expected time and place, but skeletal staining shows osteoblasts fail to mineralize the secreted matrix. This observation led us to the hypothesis that, in *her9* mutants, osteoblasts may fail to fully mature resulting in bone developmental delays and size reduction.

To understand the mechanism by which *her9* regulates bone mineralization, we have mined the literature and found a marker of late-stage osteoblasts, *spp1*, which regulates bone mineralization. To determine if osteoblasts are fully maturing, we examined *spp1* expression in *her9* mutants using RNA in situ hybridization. Preliminary results indicate that *spp1* expression in *her9* mutants is occurring at the same time and place as wild-type siblings at 5dpf fertilization. However, at 5 dpf in *her9* mutants, skeletal preps show bone has not mineralized. These results suggest that osteoblasts are fully maturing and that *spp1* is not downstream of *her9*. This preliminary data suggests that osteoblast maturation is not the mechanism responsible for the delay and reduction of bone mineralization in Her 9 mutants and motivates a non-osteoblast autonomous requirement for her9 during mineralization. In future studies, we will investigate systemic, rather than local, requirement for her9 during bone mineralization.

Funding source: NIH/NIDCR R01DE029193-02 (J.T.N.)

# 38TH ANNUAL
# RESEARCH DAY

## POST DOCS AND RESIDENTS ABSTRACTS

Friday, February 24, 2023

School of Dental Medicine
UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

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**ABSTRACTS**

**Post-Doctoral Fellows and Residents in the School of Dental Medicine**

- *Title: An ALX patterning code for the vertebrate neurocranium*.
  Authors: **Jennyfer M. Mitchell***, Tim Nguyen, Eric Van Otterloo, James T. Nichols

  **Presenting author is a Postdoctoral Fellow in the Nichols Lab, Department of Craniofacial Biology.*

- *Title: A role for the RNA modification m6A in mediating cell state switching in the fungal pathogen Candida albicans*.
  *Authors*: **Allison M. Porman Swain***, Guy G. Bushkin, Hamza Ahmed, and Aaron M. Johnson
  **Presenting author is a Postdoctoral Fellow*.

- *Title: Characterization of PDGFR$\alpha/\beta$ heterodimer-specific dynamics*.
  *Authors*: **Maria B. Campana[1*]**, Madison A. Rodgers[1], Andrew Neumann[1], and Katherine A. Fantauzzo[1]

  **Presenting author is a Postdoctoral Fellow in the Fantauzzo Lab, Department of Craniofacial Biology.*

- *Title: Prdm3 and Prdm16 genetically interact in the mammalian neural crest during craniofacial development*.
  *Authors*: **Lomeli Carpio Shull*,** Krsitin B Artinger

  **Presenting author is a Research Associate in the Artinger Lab, Department of Craniofacial Biology.*

- *PKCδ controls the DNA damage response to irradiation through regulation of chromatin accessibility and DNA double stranded break repair*.
  Authors: **Trisiani Affandi [1*]**, Ami Haas[1], Angela M. Ohm[1], Joshua C. Black[2], Gregory M. Wright[2], Jordan T. Speidel[1] and Mary E. Reyland [1]

  **Presenting author is a Postdoctoral Fellow in the Reyland Lab, Department of Craniofacial Biology.*

Category: Post-doctoral Fellows and Residents in labs at the School of Dental Medicine

Title: **An *ALX* patterning code for the vertebrate neurocranium**

Authors: **Jennyfer M. Mitchell**, Tim Nguyen, Eric Van Otterloo, James T. Nichols

**Purpose:** The study of the *ALX* gene family of transcription factors presents a unique opportunity to understand the genetic aspects that drive the development of the frontonasal region of the head.

**Methods:** In this study we used gene editing techniques to introduce mutations in zebrafish genes homologous to the *ALX* human genes to understand their function. We also used skeletal preparations and dissections to study the changes in cartilage and bone produced by these mutations in zebrafish craniofacial structures. Further, single cell RNA sequencing and multicolor whole-mount *in situ* gene expression techniques informed us about the specific zebrafish cell types that express *alx* genes. To understand the gene regulatory network surrounding *ALX* in mammals, we used chromatin immunoprecipitation and qPCR to identify upstream regulators of the *ALX* gene family.

**Results & Conclusions:** We discovered that zebrafish *alx3* functions in frontonasal neural crest cells (fNCC), to regulate distinct differentiation timing and cellular morphologies. These findings motivate the hypothesis that *alx* genes function to establish a patterning code for the neurocranium via different cellular identities among frontonasal skeletal progenitors. Here, we examine combinatorial *alx* gene expression patterns and compound mutant phenotypes to test this neurocranium patterning code hypothesis. Our gene expression studies suggest that the different subpopulations of progenitors express unique combinations of *alx* genes. Moreover, we find that different combinations of *alx* mutants produce unique skeletal phenotypes, each indicative of different identity transformations. These expression and mutant data support our identity code hypothesis. Examining our single-cell RNA sequencing dataset in search of genes expressed in the same cells as the *alx* genes, we found that the *ap2* and *alx* gene families are strongly co-expressed in zebrafish fNCC. Mutant analyses demonstrate that *ap2* function is required for correct combinatorial *alx* gene expression and that *ap2* and *alx* genes genetically interact. Finally, we discovered that in mouse Ap2 transcription factors bind to the promoter regions of *Alx* genes. These data suggest that *ap2* genes function directly upstream of *alx* genes to establish a frontonasal cellular identity code, and that this network is conserved across vertebrates.

Funding sources: NIH grants R00 DE024190-04/S1 (J.T.N.) and F32-DE029995 (J.M.M.)

Title: **A role for the RNA modification m6A in mediating cell state switching in the fungal pathogen *Candida albicans***

Authors: **Allison M. Porman Swain**, Guy G. Bushkin, Hamza Ahmed, and Aaron M. Johnson

**Purpose:** *Candida albicans* is a major fungal pathogen that causes mucosal infections such as oral thrush which can lead to deadly bloodstream infections. The goal of this research is to understand how the biology of *C. albicans* is regulated by the RNA modification $N^6$-methyladenosine (m6A).

**Methods:** To study m6A in *C. albicans*, we have constructed genetic deletions and tagged strains of the m6A methyltransferase *IME4*. RNA sequencing, m6A mapping, microscopy, and phenotypic switching analyses were completed with these strains.

**Results:** We have identified a role for *IME4* in regulating white-opaque phenotypic switching in *C. albicans*, a process that is known to regulate the ability of this species to colonize specific niches and undergo sexual mating. By performing RNA sequencing and m6A mapping in WT vs. ime4-/- strains, we have identified hundreds of genes that are differentially expressed and hundreds of sites of m6A modification. Interestingly, in contrast to the model yeast *Saccharomyces cerevisiae*, *IME4* expression is enriched in *MTL***a** and α cells and depleted in *MTL***a**/α cells.

**Conclusions:** The m6A methyltransferase *IME4* mediates switching from the opaque to white phenotypic state, which is typically considered the more pathogenic state of *C. albicans*. This switch can mediate changes in ability of *C. albicans* to form biofilms and colonize the oral niche. Future directions will investigate how ime4-/- strains are altered in their ability to colonize and infect the oral niche.

Title: **Characterization of PDGFRa/b heterodimer-specific dynamics**

Authors: **Maria B. Campana[1]**, Madison A. Rodgers[1], Andrew Neumann[1], and Katherine A. Fantauzzo[1]

[1]Department of Craniofacial Biology, School of Dental Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO

**Purpose**: The platelet-derived growth factor receptor (PDGFR) family consists of two receptors, PDGFRa and PDGFRb, that dimerize to form PDGFRa homodimers, PDGFRa/b heterodimers and PDGFRb homodimers. Signaling through the PDGFRs plays critical roles in human craniofacial development, as mutations in these receptors cause cleft lip/palate and syndromes characterized by facial dysmorphism. It has previously been impossible to study dimer-specific dynamics for the PDGFRs because commonly-used antibody-based approaches do not allow for the visualization and purification of individual PDGFR dimers. Here, we overcome these previous limitations in studying PDGFRa/b heterodimers using bimolecular fluorescence complementation (BiFC).

**Methods:** We generated and analyzed a cell line stably expressing C-terminal fusions of PDGFRa and PDGFRb with BiFC fragments corresponding to the N-terminal (V1) and C-terminal (V2) regions of the Venus fluorescent protein, respectively.

**Results:** We confirmed heterodimerization of the receptors and bimolecular fluorescence complementation upon PDGF-BB ligand stimulation of these cells via fluorescence microscopy and immunoprecipitation with a nanobody (GFP-Trap) that recognizes an epitope spanning the V1/V2 interface. We found that these receptors heterodimerize quickly in response to PDGF-BB ligand, with a peak of receptor autophosphorylation following 5 minutes of ligand stimulation. Moreover, we demonstrated that PDGFRa/b heterodimers are rapidly trafficked away from the cell membrane and into early endosomes, particularly signaling endosomes, where they dwell for extended lengths of time. Finally, we highlight current studies to identify PDGFR dimer-specific interacting proteins using mass spectrometry-based proteomics and examine the spatiotemporal activation of PDGFRa/b heterodimers *in vivo* using novel mouse models.

**Conclusions:** Collectively, our findings impart valuable insight into the molecular mechanisms by which biological specificity is introduced downstream of PDGFR activation to regulate craniofacial development.

Funding sources: NIH/NIDCR R01 DE027689-05; R01 DE027689-04S1 (K.A.F.); K02DE028572 ( K.A.F.) and the School of Dental Medicine

Title: **Prdm3 and Prdm16 genetically interact in the mammalian neural crest during craniofacial development**

Authors: **Lomeli C. Shull**, Kristin B. Artinger

**Purpose:** The gene regulatory networks and signaling pathways controlling craniofacial development must be tightly controlled, as alterations can contribute to the etiology of congenital birth defects. We previously showed that two PRDM histone methyltransferases, Prdm3 and Prdm16, facilitate cranial neural crest chondrocyte differentiation and maturation during zebrafish craniofacial development by controlling temporal and spatial Wnt/β-catenin transcriptional activity. Here, we investigated the genetic interaction between Prdm3 and Prdm16 during mouse craniofacial development.

**Methods:** *Prdm3* and *Prdm16* were conditionally deleted in the murine neural crest lineage using the Wnt1-Cre driver (*Prdm3*$^{fl/fl}$*;Wnt1-Cre*$^{+/Tg}$ and *Prdm16*$^{fl/fl}$*;Wnt1-Cre*$^{+/Tg}$). To assess whether *Prdm3* and *Prdm16* genetically interact, double floxed *Prdm3*$^{fl/fl}$*;Prdm16*$^{fl/fl}$ mice and bred them to *Prdm3*$^{fl/+}$*;Prdm16*$^{fl/+}$*;Wnt1-Cre*$^{+/Tg}$ mice to create all allelic combinatorial mutants.

**Results:** Combinatorial loss of *Prdm3* and *Prdm16* the neural crest lineage causes varying craniofacial defects, depending on the allelic combination. While loss of *Prdm3* alone causes only mild defects in mandibular hypoplasia, homozygous loss of *Prdm3* with heterozygous loss of *Prdm16* causes a highly penetrate cleft palate. Conversely, while loss of *Prdm16* alone causes mandibular hypoplasia, hypoplastic middle ear defects, with a subset of these animals developing a cleft palate, homozygous loss of *Prdm16* and heterozygous loss of *Prdm3* does not cause a cleft palate, but instead high arched palate defects and abnormal fusion of the palatal shelves. Loss of both *Prdm3* and *Prdm16* results in high-arched palate defects and abnormal palatal rugae, a hypoplastic mandible with morphological abnormalities, low set pinnae, and near complete loss of the middle ear structures. Gene expression of Wnt/β-catenin signaling components, including members of the Wnt/β-catenin enhancesome transcriptional complex, were differentially regulated in the developing facial processes of *Prdm3* and *Prdm16* mutants. Protein co-immunoprecipitations indicate Prdm3 and Prdm16 may directly interact with the Wnt/β-catenin enhanceosome complex member, Pygo2, to regulate downstream expression of Wnt/β-catenin target genes in the developing facial tissues and altering this interaction may disrupt temporal Wnt/β-catenin signaling during the development of some of these structures.

**Conclusions:** *Prdm3* and *Prdm16* have important roles in the mammalian neural crest during craniofacial development. Both factors interact with the Wnt enhanceosome during development of these facial structures. With our previous work in zebrafish, these results further suggest conserved mechanisms by which Prdm3 and Prdm16 control Wnt/β-catenin signaling during vertebrate craniofacial development.

Funding sources: NIH/NIDCR K99DE031349 (L.C.S.) and R01 DE024034 (K.B.A.)

Title: **PKCδ controls the DNA damage response to irradiation through regulation of chromatin accessibility and DNA double stranded break repair**

Authors: **Trisiani Affandi [1]**, Ami Haas[1], Angela M. Ohm[1], Joshua C. Black[2], Gregory M. Wright[2], Jordan T. Speidel[1] and Mary E. Reyland [1]

[1]Department of Craniofacial Biology, School of Dental Medicine and [2]Department of Pharmacology, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora CO 80045

**Purpose:** Patients treated with irradiation (IR) for head and neck cancer (HNC) often sustain collateral damage to non-tumor tissues in the oral cavity. Protein kinase C delta (PKCδ) regulates IR-induced apoptosis in salivary acinar cells, and inhibition of PKCδ preserves salivary gland function in mouse models of head and neck irradiation without protecting the tumor. *We hypothesize that inhibition of PKCδ suppresses apoptosis by increasing double stranded break (DSB) repair*.

**Methods:** Formation of micronuclei was quantified to investigate genomic instability. DNA damage was analyzed by γH2AX foci quantification and Comet assay. *In vivo* fluorescent reporter assay was used to directly quantify non-homologous end joining (NHEJ) and homologous recombination (HR). Chromatin remodeling was investigated using a micrococcal nuclease (MNase) assay and by analysis of histone modifications using mass spectrometry.

**Results:** RPE cells that stably overexpress PKCδ show increased chromosomal instability, indicating that PKCδ has a negative impact on genome integrity. We show that endogenous PKCδ regulates chromatin accessibility and suppresses DSB repair. Contrarily, DNA repair is increased in PKCδ-depleted cells as evidenced by more rapid resolution of IR-induced γH2AX foci and a more rapid decrease in DNA damage. Depletion of PKCδ increases DNA repair through both NHEJ and HR pathways. Depletion of PKCδ is associated with increased MNase sensitivity, suggesting a more open chromatin, while overexpression of PKCδ decreases MNase sensitivity. In PKCδ-depleted cells, H3K23ac is decreased and H3K36me2 is increased, both histone marks are associated with DNA repair.

**Conclusions:** Our data suggests a novel mechanism for control of apoptosis by PKCδ mediated through regulation of chromatin accessibility and DNA repair. Understanding the mechanism by which PKCδ regulates DNA damage-induced apoptosis may allow us to identify new targets for radioprotection of oral tissues in HNC patients.

Funding source: NIH/NIDCR R01DE027517-05 (M.R.)

38TH ANNUAL

# RESEARCH DAY

# FACULTY ABSTRACTS

Friday, February 24, 2023

School of Dental Medicine

UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

DENTAL. INTEGRATED FOR HEALTH.

## ABSTRACTS

## School of Dental Medicine Faculty

- *Title: Problem-based Learning Impact on Dental Trauma Education in Predoctoral Dental*
  *Authors*: Chaitanya P. Puranik, B.D.S., M.S., M.Dent.Sci., Ph.D.. Kaci Pickett, M.S., Tracy L. de Peralta, Ph.D, DMD, MClinEd

- *Title: Enamel Fluoride Uptake 0 to 7.5% varnish and SDF*
  *Author*: Clifton Carey, Ph.D.

- *Title: The use of surgical videos to enhance clinical periodontics teaching*
  *Authors:* Enrique Rosado, D.M.D., M.S., Jennipher Murphy, M.S., Ed.,
  Kerri Font, D.D.S, M.S.

School of Dental Medicine Faculty Abstracts

Title: **Problem-based Learning Impact on Dental Trauma Education in Predoctoral Dental**

Authors: **Chaitanya P. Puranik, B.D.S., M.S., M.Dent.Sci., Ph.D.** ,**Kaci Pickett, M.S.**, **Tracy L. de Peralta, Ph.D, DMD, MClinEd**

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**Objective**: Problem-based learning (PBL) supports education through discussion of clinical cases and promotes integration of basic and clinical sciences. The impact of PBL on dental trauma education has not been studied yet. Hence, the aim of this observational cohort study was to determine the impact of PBL on dental trauma education in a predoctoral dental curriculum.

**Methods**: Scores and first-time pass-rates in objective structured clinical examinations (OSCE) and triple jump assessments (TJA) were measured in student cohorts that did (PBL+) or did not (PBL-) receive dental trauma cases discussion in the PBL format. Student self-perceived learning outcomes were measured through a voluntary survey after competency scores were awarded. The scores and numbers of attempts on each competency were compared between the study cohorts (PBL+ or PBL-) using *t*-test and Fisher's exact tests, respectively. Mantel-Haenszel ordinal Chi-square tested for differences in rates of agreement on survey responses from students in the PBL+ or PBL- cohorts. Pearson Correlations and 95% Confidence Intervals were calculated between different competencies. The alpha value was set at 0.05.

**Results**: There was a significant improvement in the scores and first-time pass-rates in all the OSCE and TJA competencies in the PBL+ as compared to the PBL- cohort ($P < 0.05$). A higher proportion of students in the PBL+ group perceived that overall experience in dental trauma cases was beneficial as compared to the PBL- group. There was no correlation between the scores of memory-based competencies (OSCE) versus critical thinking competency (TJA) in either student cohorts.

**Conclusion**: Based on the subjective and objective evaluation, the PBL format had a positive impact on dental trauma education in a predoctoral dental curriculum.

Title: **Enamel Fluoride Uptake 0 to 7.5% varnish and SDF**

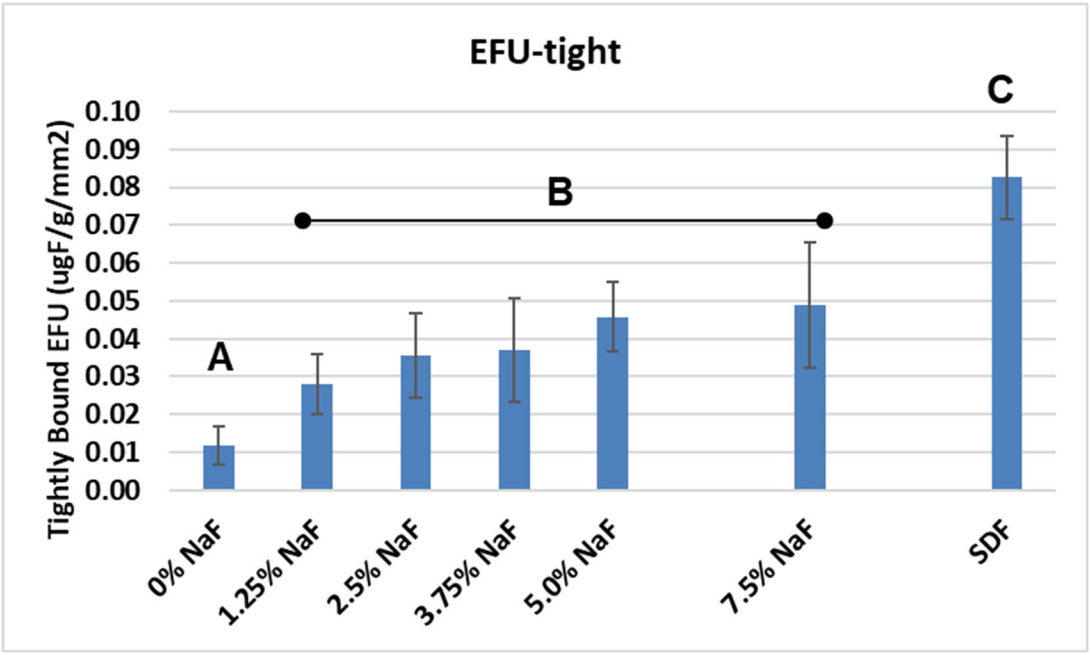
Author: **Clifton Carey, Ph.D.**

**Introduction:** The purpose of this study was to establish the relationship between the concentration of topical fluoride in varnishes and tightly bound enamel fluoride uptake (EFU-t). A second purpose was to investigate the EFU-t resulting from silver diamine fluoride (SDF) exposure. Hypotheses: (H1) EFU-t will significantly increase as varnish %NaF increases; (H2) EFU-t due to SDF will be significantly higher that EFU-t of 5%NaF varnish.

**Methods:** Enamel slabs 3x2x2mm were polished at 400grit. Baseline F content was determined via 15s $HClO_4$ extraction. Samples (n=3/group) were coated with varnish containing (0, 1.25, 2.5, 3.75, 5.0, 7.5)% NaF. SDF samples were treated with SDF solution for 2 min. All samples were soaked in F-free saliva-like solution with stirring 4h. Samples were then soaked 24h in 1M NaOH to remove loosely-bound F. Tightly-bound F was extracted by triplicate 15s extractions with 1M $HClO_4$. Extraction solutions were neutralized with 1M NaOH, TISAB added and F determined via F-ISE following ISO19448. EFU-t = ((Ext1+Ext2+Ext3)-BaselineF)/area (ugF/g/mm2) {PPM-F/mm2}.

**Results:** The were significant differences between the groups (ANOVA p=0.0001). There were no differences in EFU-t between 1.25% and 7.5% NaF groups (ranked comparisons, p>0.05). EFU-t of SDF was significantly higher than the 'plateau' varnishes (ranked comparisons, p=0.0017). The Figure shows the groups of ranked comparisons where p>0.05.

**Conclusion:** H1 is not supported as there seems to be a plateau (1.75 to 7.5%) NaF of EFU-t that is achieved where a majority of the fluoride binding sites of enamel are occupied. H2 is supported as the EFU-t is significantly enhanced by SDF.




*This study focused on tightly bound fluoride because loosely bound fluoride has no long-lasting effect on enamel protection. This is because loosely bound fluoride dissolves away into saliva (or beverages) within 24 hours. The tightly bound fluoride is persistent and will stay bound to the enamel until it is either worn off or dissolved away.*

Title: **The use of surgical videos to enhance clinical periodontics teaching**

Authors: **Enrique Rosado, D.M.D., M.S., Jennipher Murphy, M.S., Ed., Kerri Font, D.D.S, M.S.**

**Introduction:** The rapid expansion of informatics has made access to health-related information easy and expedient. A typical dental video search in the internet provides thousands of results and multiple ways to perform a procedure.

**Objectives:** The objectives of this study were to assess the use of clinical videos from the internet by dental students and residents, and to evaluate their perception about the use of institutional-created surgical videos in periodontics.

**Methods:** Clinical videos of three periodontal surgeries were created at the University of Colorado School of Dental Medicine. The surgeries included: extraction/ridge preservation, free-gingival graft, and second stage implant surgery. The procedures were recorded using a NanoCam™ surgical camera and edited using Apple iMovie® software. Microsoft Power Point™ informational slides were added to the beginning of each video in order to introduce the procedures. Questionnaires in Qualtrics XM™ were completed. An unpaired t-test ($p<0.05$) was used to determine the difference between the residents and pre-doctoral students.

**Results:** A total of 28 dental students and residents participated in the initial survey. 86% of the participants reported that they frequently access online videos to learn a dental surgical procedure. A total of 22 individuals watched the video and answered a second survey. 73% of the participants felt more confident to perform the surgical procedure after watching the video. The residents were statistically more confident after watching the video compared to dental students ($p=0.004$). 85% considered that watching the video was beneficial by supplementing lectures and helping with their clinical skills. However, statistically the most perceived benefit came from the residents ($p=0.003$).

**Conclusion:** The present study demonstrates that students are interested in viewing institutional-produced videos, and this was perceived as beneficial to their learning process.