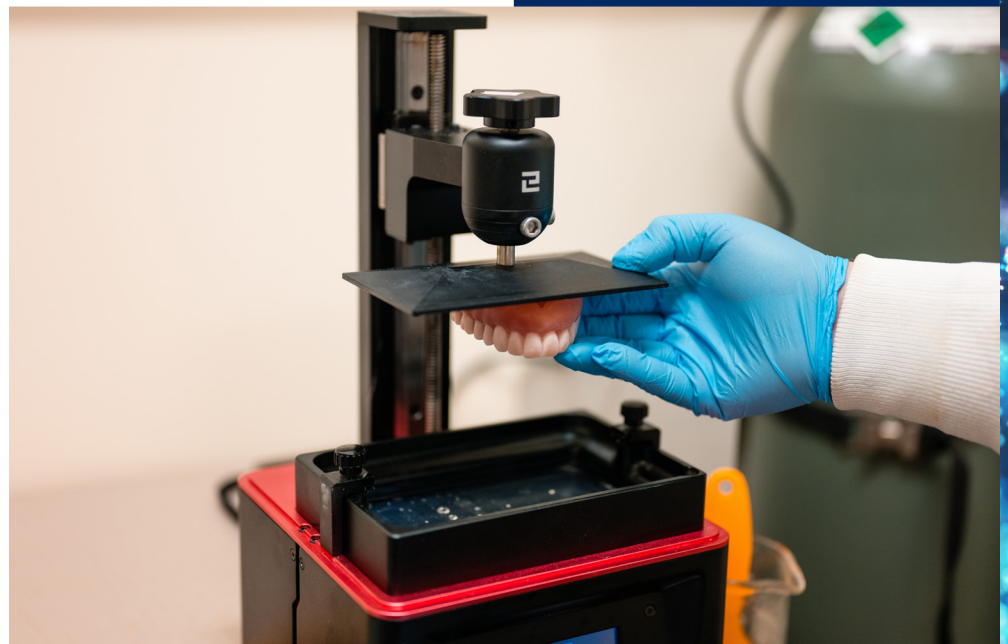# RESEARCH DAY 2025 PROGRAM

CU | School of Dental Medicine
UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS

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

DENTAL. INTEGRATED FOR HEALTH.

40th Annual Research Day
Friday, February 7, 2025
Education 2 South Auditorium and Student Bridge

8:00-8:30 am — **Poster set-up**

8:30-8:45 am — **Poster presenter briefing, judges briefing**

8:45-10:45am — **Poster viewing and judging**

10:45-11:00 am — **Travel to Auditorium**

11:00-11:05 am — **Welcome and Introduction:** Jeffrey Stansbury, PhD
Senior Associate Dean for Research and Professor, School of Dental Medicine

11:05-11:10 am — **Dean's Welcome:** Denise Kassebaum, DDS, MS, Dean, School of Dental Medicine

11:10-12:05 pm — **Keynote Speaker: David C Johnsen, DDS, MS, Professor, Dean Emeritus**
University of Iowa, College of Dentistry and Dental Clinics, Department of Pediatric Dentistry
Title: "*Critical Thinking: Learning Outcomes and Outcomes-Based Assessments*"

12:05-1:15 pm — **Lunch**

1:15-2:15 pm — **Keynote Speaker: Effie Ioannidou, DMD, MDSc, Professor**
University of California San Fransisco, School of Dentistry, Department of Orofacial Sciences
Title: "Sex and Gender in Research Methodology and Workforce"

2:15-2:30 pm — **Break and Refreshments**

2:30-2:50 pm — **Alexis Vann, DDS Student**
University of Colorado, School of Dental Medicine, Department of Craniofacial Biology *Title: "Dlx5 confines the distal cap domain during lower jaw development"*

2:50-3:10 pm — **Humberto Escobedo, PhD, Postdoctoral Research Associate**
University of Colorado, School of Dental Medicine, Department of Craniofacial Biology
Title: "Polymer Coatings that Combat Microbial Growth on Dental Materials"

3:10-3:30 pm — **Trevor Williams, PhD, Professor**
University of Colorado, Department of Craniofacial Biology, School of Dental Medicine
Title: "Craniofacial Clefting in Nepal"

3:30-4:00 pm — **Award announcements, continuing education QR-code, closing remarks**

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

DENTAL. INTEGRATED FOR HEALTH.

Thank you to our Sponsors!

centrix®
MAKING DENTISTRY EASIER®

△ **DELTA DENTAL**®

DELTA DENTAL OF COLORADO
FOUNDATION

Speaker Biographies

School of Dental Medicine

UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

40th Annual SDM Research Day 2025

Friday, February 7, 2025

Keynote Speaker

David C Johnsen, DDS, MS, Professor, Dean Emeritus

"Critical Thinking: Learning Outcomes and Outcomes-Based Assessments"

University of Iowa, College of Dentistry and Dental Clinics, Department of Pediatric Dentistry

David Johnsen received his DDS degree from the University of Michigan in 1970 and his MS in Pediatric Dentistry from the University of Iowa in 1973.He became board certified in Pediatric Dentistry in 1978. Professor Johnsen has been on the faculties of West Virginia University from 1974-80, with the Outstanding Teacher Award, 1976; Case Western Reserve University from 1980-95, where hwhere he served as Department Chair, intermittently as Director of the Residency Program, and as Interim Dean in 1993; and served as Dean of Dentistry at the University of Iowa from 1995 to 2021. He is now Dean Emeritus and Professor of Pediatric Dentistry at the University of Iowa.

Professor Johnsen's research and scholarship have focused in three main areas, two with federal funding: Innervation of teeth as an indicator of capacity to transmit pain sensory impulses (NIH) and early childhood caries/caries patterns in preschoolers (MCH). The latter area included demonstration projects or consulting nationally in the Women, Infant, and Children's (WIC) Program and the Head Start Program. Current activity is in the methodology for critical thinking learning and assessment; he is in the top 1% of scholars writing about Dental Education over the past 10 years. He has also published on a variety of clinical and educational topicsProfessional service has included the American Academy of Pediatric Dentistry Board of Directors, 1988-91 and Child Health (legislative) Advocate, 1992-95, with the Distinguished Service Award in 1996. Dr. Johnsen received the Achievement Award from the Ohio Dental Association in 1992. He served on the American Dental Education Association Council of Deans Board, 1998-2001 and as ADEA President, 2002-2003. He received the ADEA Distinguished Service Award in 2021. He received the Jack Hein Public Service Award from the American Association for Dental Research in 2010. He served (2010-13) on the National Advisory Dental and Craniofacial Research Council (NIH). He was Director of the Dental Deans Institute from 2014 - 2021. He is currently on the Editorial Board for the Journal of the American Dental Association.

University Service has included: Election to Chair the Faculty Senate at Case Western Reserve University, 1994. At the University of Iowa, he served as Chair of the Search for the University of Iowa Hospitals and Clinics CEO, 2002; as Co-Convener of the Health Sciences Policy Council, 2003-04; as Co-Chair of the University Task Force on Clinical/Translational Research in 2005-2006; as Chair of the Nursing Dean Search in 2006; as Chair of the Presidential Search in 2007; as Chair of the UI Museum of Art Director Search in 2010; and as Chair of the Liberal Arts and Sciences Dean Search in 2012. Thirteen professorships created. 2007-2016: $65million Building Transformation completed on time and under budget.

40th Annual SDM Research Day 2025

Friday, February 7, 2025

Keynote Speaker Cont.

David C Johnsen, DDS, MS, Professor, Dean Emeritus

"Critical Thinking: Learning Outcomes and Outcomes-Based Assessments"

University of Iowa, College of Dentistry and Dental Clinics, Department of Pediatric Dentistry

Dr. Johnsen's presentation will discuss how critical thinking is an essential skill and an essential component for the successful practice of dentistry. There is no national consensus on defining the learning outcome, guiding learning, and assessing performance for critical thinking skills. The literature is sparse on learning outcomes for critical thinking skills, sparser on outcomes-based assessment of critical thinking. A basic premise is that a designated thought process is the learning outcome, the learning guide, and the assessment instrument. The goal is to guide what the student is to do in their thought processes- specifically to demonstrate the designated thought process with their patient. The umbrella term "patient-based, student-led demonstrations of thinking and judgment" was selected to try to encompass the theme of the collection of exercises. Each of the exercises is patient or case based; "student led" is key in critical thinking demonstration with the student applying the designated thought process to the patient or case; in each of the exercises, the student applies a designated thought process to a patient/case; in each case, structured thinking and judgment are called for as there are no preconceived "right answers." Assessment is based on mastery of the thought process as applied to the patient or case. Examples of student demonstrations of thinking and judgment will be presented.

40th Annual SDM Research Day 2025

Friday, February 7, 2025

Keynote Speaker

Effie Ioannidou, DMD, MDSc, Professor

"Sex and Gender in Research Methodology and Workforce"

University of California San Fransisco, School of Dentistry, Department of Orofacial Sciences

Dr. Effie Ioannidou is a clinician scientist with training in Periodontology. Her research addresses clinical and translational questions on the anti-inflammatory effect of periodontal interventions in systemic health and chronic kidney disease. She has recently led a multicenter team of investigators to explore systemic antibiotic prescribing patterns in combination with periodontal therapy. By leveraging advanced molecular techniques, her team aims to advance the field and provide evidence-based guidance for clinicians, ultimately leading to more effective and sustainable periodontal treatment outcomes. By addressing the antibiotic stewardship priorities outlined by the NIH and the CDC, her lab produced preliminary data suggesting that antibiotic treatment leads to a microbial shift towards healthier communities, supporting the underlying hypothesis. Her team recently showed that systemic antibiotics are widely prescribed during initial periodontal treatment without clinical practice guidelines related to adjunct antibiotics. Recently, her team developed a new line of research focusing on the intersection of sex and gender in oral inflammatory diseases. They have produced data that highlights the lack of adherence to reporting guidelines as it pertains to sex and gender in RCTs in periodontology. Therefore, her lab is focusing on better understanding sexual dimorphism in periodontitis to explain disease disparities. Dr. Ioannidou is passionate about rigor and transparency in clinical research and proponent of high-quality scientific reporting and clinical trial registration.

Dr. Ioannidou is a founding member of the IADR Women in Science Network. She served as the Associate Editor of the JDR Clinical and Translational Research until June 2023. She is currently the AADOCR President-elect (2022-2026). She has served as an ADA Council on Scientific Affairs member. She is the Task Force on Design and Analysis in Oral Health Research's Executive Director on Design and Analysis in Oral Health Research. She is an AADOCR (2018) and AAAS (2022) Fellow. Since July 1st, 2023, Dr. Ioannidou has served as the Editor-in-Chief of the two AAP Journals: Journal of Periodontology and the Clinical Advances in Periodontics

Dr. Ioannidou's lecture aims to first establish the correct terms of sex biology and gender construct. Historical perspective and the old and new paradigms will be discussed. Her goal is to help atendees understand the role of sex and gender in research methodology and workforce.

40th Annual SDM Research Day 2025

Friday, February 7, 2025
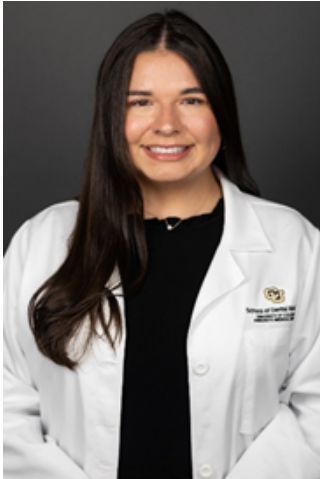
Alexis Vann, DDS Student

"Dlx5 confines the distal cap domain during lower jaw development"

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

Alexis completed my undergraduate education at the University of Central Arkansas. It was there that she first performed research, working under the direction of her faculty mentor, Dr. Mick Yoder, Assistant Professor of Biology. In Dr. Yoder's laboratory she was able to gain insight into the world of embryogenesis and developmental biology; Alexis valued the research and learning experiences that she was able to gain under the mentorship of Dr.Yoder. This experience led to an appreciation for research that she did not previously have.

After graduating, Alexis was accepted in the Doctor of Dental Surgery program at the School of Dental Medicine, University of Colorado Anschutz Medical Campus (CU AMC). While it was not the only school into which she was accepted, The Summer Scholars Program was a large reason that CU SDM was my top choice for the school at which to complete my dental education. As a pre-first-year dental student at CU AMC, she had the opportunity to continue my research journey in Dr. David Clouthier's Lab. This time Alexis was able to combine her interest in research and her love for the dental field by exploring aspects of oral cavity development. Her project explored the function of genes that are hypothesized to influence the development of the lower jaw. Understanding why developmental differences in humans occurs will aid in developing better treatments, though interrogating gene function in mice is necessary in order to understand what occurs in humans. Her project focused on the role that the transcription factor Dlx5 regulates a signaling domain associated with incisor development. Alexis' main goal was not to only complete her project, but to begin to integrate applied science and evidence-based reasoning into her career as a dentist. The field of research in the world of dentistry is just as important as the dentistry itself. Without research there is no world in which dentistry can transform lives of patients.

Alexis' presentation will cover the background of pharyngeal arch development and how Dlx5 is first expressed and then repressed. She will then cover how we overexpressed Dlx5 in the distal arch and the outcomes of this overexpression, which include retrognathia and tooth and tongue defects. Alexis will also describe some of the gene expression changes that accompany overexpression, and she will end with a summary and model.

CU School of Dental Medicine
UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS

DENTAL. INTEGRATED FOR HEALTH.

40th Annual SDM Research Day 2025

Friday, February 7, 2025

Humberto Escobedo, PhD, Postdoctoral Research Associate

"Polymer Coatings that Combat Microbial Growth on Dental Materials"

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

Humberto Escobedo, PhD, is a postdoctoral research scientist in Dr. Devatha Nair's lab at the Department of Craniofacial Biology. He earned his PhD in Pharmaceutical Sciences from the University of Colorado Anschutz Medical Campus, Skaggs School of Pharmacy and Pharmaceutical Sciences. Dr. Escobedo's research focuses on studying the impact of incorporating azo compounds in polymer-based coatings and in bulk materials that combat microbial growth.

Dr. Escobedo's presentation will discuss how in the US, there are 120M adults who are missing at least one tooth and 36M are completely edentulous with many going untreated for lack of affordable options. Even when affordable, dentures have been known to selectively increase certain microflora and/or increase the total number of microorganisms in the microbiome, which in turn are risk factors for multiple systemic diseases. This leads to serious quality of life and overall general health consequences. Here we present 3D-printable resins for dentures with substantial improvements in the material properties including enhanced strength, stiffness, toughness and wear resistance. Additionally, we incorporate antibacterial monomers within dentures to specifically inhibit Streptococci spp and have the demonstrated ability to be transiently mobilized by light to disrupt biofilms growing on their surfaces. With dentists becoming increasingly involved in the management of systemic health issues, there is a need to learn and embrace platforms that offer affordability for patients most in need while also significantly raising the clinical value and performance over the entire market.

Dental materials, such as dentures, are used to restore function and confidence in individuals. However, improper hygiene along with conventional materials can lead to oral complications such as tooth decay, gum disease, yeast infections and pneumonia. Here we investigate the potential benefits of incorporating acrylated hydroxy azobenzene (AHA) in denture-based resins to inhibit oral infections such as denture stomatitis to advance dental healthcare in patients who otherwise find it difficult to maintain proper oral hygiene.

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

DENTAL. INTEGRATED FOR HEALTH.

40th Annual SDM Research Day 2025

Friday, February 7, 2025

Trevor Williams, PhD, Professor

"Craniofacial Clefting in Nepal"

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

Trevor Williams is a Research Scientist in Molecular Genetics at the University of Colorado, Anschutz Medical Campus in the Department of Craniofacial Biology. He obtained his undergraduate degree from Trinity College, Cambridge University in the UK in 1981, specializing in pathology.. Later, in 1986, he earned his Ph.D. from Imperial College, London, where he studied the interactions between viruses and the genetic material present in

mice and humans. He then moved to the University of California, Berkeley, for his post-doctoral studies, where he focused on a specific group of mammalian genes known as the AP-2 transcription factors. Transcription factors ensure that the right genes are activated in the right place and at the right time and he later showed AP-2 family was critical for face and tooth development. Subsequently, he took this project with him to his faculty position at Yale University and later at the University of Colorado, where he runs a basic research laboratory studying the genetics of craniofacial development. In 2023, Dr. Williams received a 6 month Fulbright U.S. Scholarship to work at Kathmandu University in Nepal while attached to the Dental School of Dhulikhel Hospital. His ongoing studies in Nepal concern the demographics of orofacial clefting in Nepal, the history of treatment in the country, and the factors behind patient decisions to receive or decline treatment.

Dr. Williams' lecture will discuss the progress of studies begun during a 6-month Fullbright Scholarship to Nepal. More specifically, he will discuss how the causes of facial clefting are influenced by multiple factors, including environmental and genetic. Seeking appropriate support and medical treatment depends on cost, access, and cultural concerns, and such factors need to be considered when treating any patients with cleft lip+/- palate.

#Poster	Name:	TITLE:
1DDS/ISP	NOT PRESENTING	
2DDS/ISP	Noah Booth	Annotated Radiographic Analysis using Artificial Intelligence Compared to Oral and Maxillofacial Surgeons Interpretation of Impacted 3rd Molars
3DDS/ISP	Eirini Boutiou	Early Prosthetic Management of Oligodontia in Ectodermal Dysplasia
4DDS/ISP	Rubi Diaz Delgado	Engaging Students in Higher Order Thinking to Enhance Learning Using Think-Pair-Share
5DDS/ISP	Kylee Fulton	Inhibition of Streptococcus mutans through Hydroxylated Azobenzene Compounds as applied to 3D Printed Dentures
6DDS/ISP	Emma Heiny	Craniofacial and dental phenotypes in a zebrafish model of Polr3-related disease
7DDS/ISP	Ana Horvath	Using experiential learning techniques to improve confidence in intraoral scanning
8DDS/ISP	Mackenzie Korbel	Changes in Dental Students' Empathy Levels
9DDS/ISP	Woohykeok Kwon	Digital vs. Conventional Splint Fabrication: Assessing Fit, Contacts, and Time Efficiency
10DDS/ISP	Brittany Meola	Reduction of Stain Caused by SDF Therapy
11DDS/ISP	Nisali Piyasena	Assessing the Mechanism of Streptococcus Inhibition by Azobenzene Copolymers
12DDS/ISP	Edwin Sarkisians	A Photographic Method to Estimate Shade Change Due to Bleaching
13DDS/ISP	Gosia Waz	Dental Students' Perceptions on International Service-Learning Activities at a US and Brazilian dental school
14DDS/ISP	Kamden Hilborn	Impact of Manikin-type on Preclinical Exercises in Predoctoral Pediatric Dentistry
15P	Bracken Bateman	Pain, efficacy and preference associated with needle-free anesthetic injections compared to needle injections for orthodontic TAD placement: a prospective randomized clinical trial.
16P	Evan C. Brooks	Differential Srsf3 protein interactions upon PDGFRa signaling in mouse embryonic palatal mesenchyme
17P	Maria Campana	PDGFRa/b heterodimer activation negatively affects downstream ERK1/2 signaling
18P	Morgan Lane	Pain control in orthodontic patients using combined acetaminophen and ibuprofen compared to ibuprofen alone or placebo: a single-center, blinded, randomized, controlled, clinical trial.
19P	Elaine Lee	Comparison of self-reported pain associated with initial placement of conventional, superelastic, or thermoelastic nickel-titanium archwires: a blinded, controlled, randomized clinical trial.
20P	Hanna Lee	Development of a method for age estimation for adults using a CBCT derived pulp/tooth area ratio method: a controlled retrospective, observational study.
21P	Stanley Kanai	Investigating the role of intracellular Ca^{2+} dynamics in lower jaw development
22P	Sujeong Lee	A comparison of orthodontic miniscrew stability and peri-implant inflammation in orthodontic patients when miniscrews are precoated with antimicrobial quaternary ammonium compound K21 or ethanol control on the surface: a triple-blind, split-mouth, randomized controlled clinical trial.

23P	Jereme Sebastian	Use of toothpastes containing non-traditional ingredients including activated charcoal, nano-hydroxyapatite, potassium nitrate, calcium carbonate, coconut oil, 5% hydrogen peroxide, or without fluoride prior to bonding on orthodontic bracket bond strength in the shear mode; a controlled in vitro study.
24P	Colt Sicher	The effect of vibration therapy versus no vibration therapy on clear aligner sequential fit in orthodontic patients: a single center, prospective, randomized controlled clinical trial.
25P	Menaka Tandon	Comparing 3D changes in volume of the nasal cavity via cone-beam computed tomography (CBCT) in patients undergoing maxillary skeletal expansion: a prospective clinical pilot trial.
26P	Tahina Torres	Identification of WSLs in orthodontic patients with a novel nano-particle fluorescent caries detection system compared to visual detection: a controlled clinical trial.
27P	Dianzheng Zhao	A new model of cleft lip and palate demonstrates aberrant Notch signaling alters periderm formation and Wnt signaling dynamics
28P	Alejandro Arango	Enamel damage after debonding monocrystalline ceramic brackets compared to debonding metal brackets
29P	Raisa Bailon-Zambrano	A Closer Inspection at the Vertebrate Head Reveals Ancestral Patterning Domains
30S	Erin Binne	Characterization of the role of Srsf3 in the mouse facial ectoderm
31S	Christine Forrester	A LOOK INSIDE DENTAL HIGH-VOLUME EVACUATION LINES
32S	Anna Gartner	Properties of monourethane (meth)acrylates when used as low viscosity reactive diluents
33S	Austyn Salazar	Formulating Photopolymer Systems for Multimaterial Jetting with a Range of Mechanical Properties
34S	Colette Dolby	*prrx1* genes regulate developmental timing of median fins
35S	Bailey Lubash	POLR3A is required for craniofacial cartilage and bone development
36S	Sebastian Lopez	Light-Propelled Azobenzene Nanogel for Dental Adhesive
37G	Maggie Keating	Investigating the developmental origins of median fins through mutant analysis
38G	Grace Gustafson	Investigating a new buffering mechanism for overcoming deleterious mutations
39G	Gabriela Padilla	Identifying the subcellular compartment(s) that serves as the major signaling platform for PDGFR homodimers and heterodimers
40G	Charles Griffin	Characterizing the role of Akt-mediated phosphorylation of Srsf3 during mouse craniofacial development
41G	Cassie Minne	Alternative RNA splicing of transcripts encoding protein serine/threonine kinases
42G	Abi Mumme-Monheit	Variable paralog expression underlies incomplete penetrance
43G	Jared Ortiz	AI Periapical Detection
44G	Nadia Wright	*alx* function in the frontonasal skeleton revives the pharyngeal arch-0 hypothesis.
45F	Tanya Wright, DDS	Active Learning: Encouraging Pathology Integrations Through Patient Box Questions
46F	Cori Hendricks /Yanira Owens	Empowering the Utility of Peer Teaching in Pre-clinical and Clinical Education
48F	Catherine Flaitz	Increasing Student Engagement in General Pathology with Two Teaching Tools
47F	Susan Somerset	Aged out and Lost! Establishing a Dental Home for Adults with Special Healthcare Needs

DDS-ISP Abstracts

School of Dental Medicine

UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

Title: Annotated Radiographic Analysis using Artificial Intelligence Compared
to Oral and Maxillofacial Surgeons Interpretation of Impacted 3rd Molars

Authors: William McMunn III DDS MD FACS, Benjamin Crockett DMD MS, Michael Lampe Ed.D, James J. Closmann DDS, Mudit Nahata MS, Noah Booth

Category: Dental students and ISP students at the School of Dental Medicine

Introduction/Background: The Overjet artificial intelligence model demonstrates a substantial level of agreement with Oral Surgeons in assessing percent of third molar impaction from panoramic radiographs. This finding supports the use of Overjet in clinical settings with the potential to reduce subjective bias and improve coding speed and accuracy.

Traditionally, Oral Surgeons have relied on their own evaluation of panoramic radiographs to determine the position and impaction status of third molars, assigning ADA codes to guide extraction procedures and assessing those codes based on clinical findings at the time of surgery. However, the inherently subjective nature of these radiographic assessments can lead to variability in diagnoses and subsequent coding. To address this variability, recent advancements in AI models, such as Overjet, have been developed to evaluate the impaction status of third molars in an objective manner. Oral and maxillofacial surgery stands to benefit enormously from the continued and careful integration of AI into clinical practice, provided that AI advancements can be proved reliable.

Aim: To compare the predicted degree of impaction (non-impacted, partial bony or full bony) assigned by two Oral Surgeons with those assigned by Overjet AI for third molar extractions

Methods: Using panoramic radiographs, 60 maxillary molars and 120 mandibular molars were reviewed by two Oral Surgeons as well as Overjet AI. Oral Surgeons independently assigned one of the following to each 3rd molar:
- Non-impacted: none of the crown is covered by bone
- Partial bony impaction: part of crown covered by bone (< 50%)
- Full bony impaction: most or all of crown covered by bone (≥ 50%)

Percentage of impaction as assessed by Overjet AI was converted as follows:
- 0-15% = *non-impacted*
- 16-50% = *partial bony impaction*
- 51-100% = *full bony impaction*

Outcomes/Results:

Reliability**:**

Cohen's kappa for observer 1 vs observer 2:
- Maxillary molars 0.798 (substantial agreement)
- Mandibular molars = 0.848 (Almost perfect agreement)

Fleiss' kappa:
- Maxillary molars 0.707 (substantial agreement)
- Mandibular molars = 0.693 (substantial agreement)

Accuracy**:**

Maxilla
- AI vs Observer 1
- No Impaction = 100.00
- Partial Bony = 37.50
- Full Bony = 90.90
- AI vs Observer 12
  - No Impaction = 100.00
  - Partial Bony = 46.67
  - Full Bony = 93.94

Mandible
- AI vs Observer 1
  - No Impaction = 54.17
  - Partial Bony = 61.76
  - Full Bony = 95.08
- AI vs Observer 2
  - No Impaction = 55.00
  - Partial Bony = 60.00
  - Full Bony = 93.75

Conclusion:

The Overjet artificial intelligence model demonstrates a substantial level of agreement with Oral Surgeons in assessing percent of third molar impaction from panoramic radiographs. Further studies incorporating three-dimensional data are essential to enhance the correlation between panoramic and clinical evaluations.

Title: Early Prosthetic Management of Oligodontia in Ectodermal Dysplasia

Authors: Boutiou E, Anderson N, Nguyen P, Chiquet B, Acharya B
University of Colorado, Aurora, CO
University of Texas Health Center Houston

Category: Dental students and ISP students at the School of Dental Medicine

Ectodermal dysplasia is a rare genetic disorder occurring in 1 in 100,000 live births that can be X-linked, autosomal dominant, or autosomal recessive. This condition causes a malformation in two or more ectodermal derivatives including teeth, hair, nails, and sweat glands, with some individuals also presenting with cleft lip and/or palate. AMected individuals often experience disturbances in tooth development, which may result in oligodontia, hypodontia, defects in tooth eruption, and abnormal variations in tooth size and shape. This case report highlights the fabrication of a removable jackscrew-activated maxillary prosthesis designed to establish function, esthetics, and phonetics in a patient with oligodontia. Additionally, it emphasizes the importance of early intervention as it can significantly impact the emotional and psychological development of patients in school age.

Title: Engaging Students in Higher Order Thinking to Enhance Learning Using Think-Pair-Share

Authors: Pramod Philip, John Kharouf, Jennipher Murphy, **Rubi Diaz Delgado***

Category: Dental students and ISP students at the School of Dental Medicine

Abstract: Lecture is a primarily one-way dissemination of information with students listening, taking notes and engaging content through quizzes. Research demonstrates that integrating active learning strategies in lecture courses can increase engagement and enhance learning. The Think-Pair-Share strategy is an intentional constructivist activity and an active learning technique where students transfer and apply their foundational knowledge and cognitive process to new scenarios or by tackling problems. The goal of this study was to improve student engagement and enhance learning for second-year dental students taking the Orthodontics I course. This didactic course is taught in a lecture format with no opportunities to practice hands-on orthodontic or problem-solving skills. The course was modified to include opportunities for students to practice critical thinking and higher-level cognitive skills through hands-on practice, think-pair-share (TPS) activities, and formative self-assessments. TPS activities were focused on reinforcing concepts that students have historically struggled to grasp. These activities were presented in a low-stake environment and provided an opportunity to provide feedback to students in real time. The TPS activities had no impact on students' grades, but the formative self-assessments counted as bonus points, providing an opportunity for a boost in their grades but not subtracting or lowering their grades. A non- mandatory survey was sent to students before the midterm to obtain feedback about the new Orthodontics I course class format. Of the 79 students enrolled, 17% participated in the survey, 57% of these agreed/somewhat agreed that the TPS activities were helpful for their learning or understanding of the material, and 71% reported being fully or mostly engaged during the in-class activity. Our study shows a positive response towards the in-class activities, voluntary engagement from the students, and increased attendance. However, when comparing the performance of students during the Orthodontics I midterm with that of students during a previous year, there was not an overall improvement observed. This comparison suggests that our novel approach to the Orthodontics I course may not be beneficial for improving learning and retention.

Project Title: Inhibition of *Streptococcus mutans* through Hydroxylated Azobenzene Compounds as applied to 3D Printed Dentures

Authors/Investigators: Kylee Fulton, Humberto Escobedo, Austyn Salazar, David Danforth, Jeff Stansbury, Michael Schurr, Devatha Nair

Category: Dental students and ISP students at the School of Dental Medicine
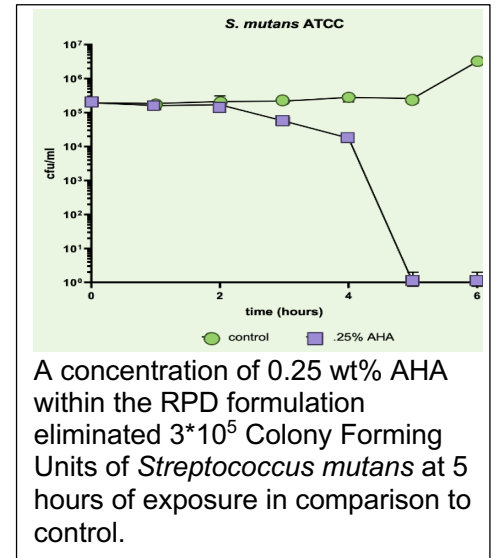
Background

Despite advances in 3D printing and dentistry, the fabrication of robust long-lived components of dentures remains an unmet goal. Dentures can harbor *Streptococcus mutans* and other bacteria, causing high rates of oral cavity infections among wearers. This study examines a novel formulation for a 3D-printed removable partial denture (RPD) base with outstanding mechanical properties and targeted antibacterial effect.

Methods

Concentrations of acrylated hydroxyazobenzene (AHA) ranging from 0-2% weight were added to a resin of 46% urethane dimethacrylate, 37% ethylhexyl acrylate, and 17% methacrylic acid and polymerized using a redox reaction and thermal post-cure. Samples were subjected to 3-point bend testing in dry and wet conditions to determine flexural strength and modulus. *S. mutans* was grown on BHI plates and in liquid culture and was subsequently exposed to AHA-containing resin pucks to assess inhibition.

Results

RPD with 0.25 wt% AHA achieved high double-bond conversion in comparison to the control (RPD=97.4 ± 0.62% vs. RPD+AHA=97.2 ± 0.60%). The mechanical properties of the RPD + 0.25 wt% AHA were comparable to that of the control for modulus measurements (RPD = 1.59±0.08 GPa vs. RPD +AHA= 1.57±0.14 GPa) and flexural strength tests (RPD= 79.1 ±6.0 MPa vs RPD+AHA= 70.9±3.9 MPa), indicating that the RPD and RPD+AHA meet the ISO standards for denture materials. At 0.25 wt%, the AHA also inhibits the growth and proliferation of S. mutans (Figure 1).

A concentration of 0.25 wt% AHA within the RPD formulation eliminated $3*10^5$ Colony Forming Units of *Streptococcus mutans* at 5 hours of exposure in comparison to control.

Conclusions

The RPD formulation with *Streptococci*-specific inhibitory additive AHA maintained its high strength attributes at a 0.25 wt% of the AHA. Future studies will test AHA against pathogenic bacteria such as *Streptococci pneumoniae*. CU SM will use the AHA-modified RPD base in multimaterial inkjet printing of monolithic RPD base and tooth prostheses for eventual application in case studies and clinical trials following appropriate clearances.

Project Title: Craniofacial and dental phenotypes in a zebrafish model of Polr3-related disease

Authors/Investigators: Emma Heiny, Bailey Lubash, Kristin Watt

Category: Dental students and ISP students at the School of Dental Medicine

SEE POSTER PRESENTATION - VIEWING ONLY

Title: Impact of Manikin-type on Preclinical Exercises in Predoctoral Pediatric Dentistry

Authors: Hilborn K*[1], Cartwright B[1], Cabana K[1], Pickett-Nairne K[2], Nguyen P[3], Puranik CP[4]

Affiliations
* Presenting author

1 Doctor of Dental Surgery Candidate, University of Colorado School of Dental Medicine, Aurora, CO

2 Kaci Pickett-Nairne, MS, Research Instructor, Center for Research in Outcomes for Children's Surgery, Children's Hospital Colorado, University of Colorado School of Medicine

3 Paloma R. Nguyen, D.M.D., M.S.D, Diplomate, Assistant Professor, Director of Predoctoral Education in Pediatric Dentistry University of Colorado School of Dental Medicine, Aurora, CO 4 Chaitanya P. Puranik, B.D.S., M.S., M.Dent.Sci., Ph. D. F.A.A.P.D., Diplomate, Associate Professor, Director of Residency Program in Pediatric Dentistry Children's Hospital Colorado and University of Colorado School of Dental Medicine, Aurora, CO

Category: Dental students and ISP students at the School of Dental Medicine

Purpose: Most preclinical pediatric dentistry programs use standard adult manikins with pediatric typodonts during preclinical pediatric dentistry exercises. In contrast to adult, the pediatric manikin provides limited access for simulated pediatric restorative procedures. The purpose of this study was to comparatively evaluate the impact of manikin (adult vs. pediatric) on student's performance and evaluation in a preclinical pediatric dentistry course.

Methods: This observational cohort study evaluated Class II tooth preparations and stainless-steel crowns (SSC) in a predoctoral pediatric dentistry course (N=160). Both preparations were performed with pediatric mixed dentition typodonts with two types of manikins: adult vs. pediatric (independent variable). Student and faculty graded performance using predetermined parameters and rubrics. Grading criteria (met vs. not met) and student-faculty agreement were the two dependent variables. Differences in results by manikin type were compared with Wilcoxon Rank- Sum tests (α=0.05).

Results: There were 18 and 10 evaluation parameters for Class II and SSC preparations, respectively. A higher proportion of students met all grading criteria for SSC and Class II with use of adult manikin as compared to pediatric manikin (P<.001). Conversely, a higher proportion of students had critical errors in their SSC and Class II preparations when pediatric manikins were used (P=.001). There was a statistically higher agreement between student-faculty evaluation for SSC and Class II when adult manikin was used as opposed to pediatric manikin (P<.001).

Conclusions: Limited access with pediatric manikins increased the challenges associated with preclinical pediatric dental exercises emphasizing the value of pediatric manikins in preclinical pediatric dental education.

Title: Using experiential learning techniques to improve confidence in intraoral scanning

Authors: Anastasia Horvath

Category: DDS/ISP Dental Student

This study aimed to evaluate the effectiveness of the experiential learning model in improving confidence in the use of intraoral scanning technology among faculty members in the Restorative Dentistry department. Thirty-four faculty volunteers participated in a digital dentistry training course focused on the Trios 5 intraoral scanner. The training included a presentation, a demonstration on proper scanner use, and hands-on practice in pairs. Pre- and post-training surveys were administered to assess changes in participants' confidence levels regarding their ability to use the intraoral scanner. The post-survey also evaluated the retention of critical information related to the operation of the Trios 5 scanner. Results indicate that faculty members reported improvements in their confidence in using the scanner after the training, and demonstrated strong retention of key Trios 5 information. These findings suggest that the experiential learning approach—combining instruction with active practice—can be an effective method for enhancing faculty proficiency and confidence in digital dentistry tools, particularly intraoral scanners.

Title: Changes in Dental Students' Empathy Levels

Authors/Investigators: Tippets, J, Wilhelm, K, McMunn, W, Renaud, D, Houliston, C, **Korbel, M,** & Murphy, J.

Category: Dental students and ISP students at the School of Dental Medicine

Objectives:

Affective, or emotional, empathy is the ability to share the same emotions with other individuals. Within dentistry, emotional empathy can help to build personal connections between patients and providers, but can also lead the providers to experience distress, as they become affected by the negative experiences of their patients. When developing into a dental care provider, dental students must consider the cost of emotionally connecting with their patients and protecting their own well-being. Current research suggests that there is a decline in emotional empathy in dental students over their four years in dental school, but little data demonstrates at which point in the student's education this decline occurs. This study aims to determine if there is a difference between empathy levels in students before and after transitioning from the classroom to the clinical setting.

Methods:

The Toronto Empathy Questionnaire (TEQ) was administered to pre-clinical second-year and clinical third-year dental students in a classroom setting. The TEQ was chosen for use due to its evidence-based validity and reliability, and its ability to provide numerical data on dental students' empathy levels at a given point in time. The average scores of the pre-clinical second-year students and the clinical third-year students were then compared. Additionally, the current third-year students' average score from this year was compared to last year to evaluate longitudinal data.

Results:

Data analysis for the study will be completed in January.

Conclusions:

This study was designed to be repeatable to evaluate students at various stages in their education, specifically during the transition from the classroom to the clinical setting. Future extensions of this study include exploring situational-based evaluations of students' empathy. We hope to use this research to gather comprehensive insights into empathy development among dental students.

Project Title: Digital vs. Conventional Splint Fabrication: Assessing Fit, Contacts, and Time Efficiency

Authors: K. Baskaran, D. Gozalo, J. Murphy, **W. Kwon***

Category: Dental students and ISP students at the School of Dental Medicine

Abstract: Digital splint fabrication demonstrates significant advantages in speed and cost while maintaining precision comparable to conventional methods. With further refinement, it holds promise as a superior clinical option.

Introduction/Background: Splints are essential in managing occlusal and temporomandibular joint disorders (TMD). Conventional fabrication methods are time-intensive, operator-dependent, and prone to inconsistencies. Digital fabrication offers a potentially faster, more precise, and consistent alternative. This study compares digital (Method B) and conventional (Method A) splint fabrication in cost, fit, occlusion accuracy, fabrication time, and adjustment time.

Aim: Digital fabrication (Method B) was hypothesized to outperform conventional fabrication (Method A) in cost, fit, contact accuracy, and fabrication time, with comparable adjustment time.

Methods: Ten Kilgore 200-series hard tissue models were used. Each model received two splints: one digital (Method B) and one conventional (Method A). Digital splints were designed using 3Shape Splint Design Studio software, scanned with 3Shape TRIOS 5, and 3D- printed with Formlabs Form 3B printer and Dental LT Clear Resin. Conventional splints involved alginate impressions, Type IV dental stone models, and laboratory fabrication. Key metrics included cost, fit, occlusal contacts, fabrication time, adjustment time, and total score. Descriptive statistics and independent t-tests were applied.

Outcomes/Results:

- **Fit:** Method B had a higher mean score (4.70 vs. 3.80, p = 0.122).

- **Contact Accuracy:** Comparable in CR (p = 0.861) and excursive movements (p= 0.315).

- **Intensity of Contact:** No significant difference (p = 0.571).

- **Fabrication Time:** Method B was significantly faster (4.20 vs. 1.70, p < 0.001).

- **Adjustment Time:** Method B required slightly more time (2.30 vs. 1.50, p = 0.079).

- **Cost:** Method B was substantially cheaper ($9 vs. $131).

- **Total Score:** Method B scored higher (3.02 vs. 2.20, p = 0.07).

Conclusion: Digital splints demonstrate efficiency, cost-effectiveness, and comparable performance. Minor discrepancies in occlusal contact suggest further research into 3D printing material shrinkage to enhance precision.

Title: Reduction of Stain Caused by SDF Therapy

Author: Brittany Meola and Clifton Carey

Category: Dental students and ISP students at the School of Dental Medicine

Introduction: One of the main side effects of SDF is that over a 2-week period the application site will turn black in color that is persistent for years. Many practitioners will therefore not use SDF to arrest caries on anterior teeth.

Objective: The objective of the project was to develop a method to reduce the stain caused by silver diamine fluoride (SDF) treatment. The hypothesis was that citric acid saturated with respect to hydroxyapatite will selectively chelate silver from the treated enamel and dentin surfaces, thereby significantly reducing the stain.

Experimental method: Dentin samples (n=34) were exposed to SDF to develop commonly observed dark stains. Digital images were taken of all samples for baseline color stain with a reference white in each photograph. Samples (n=17) were assigned to two groups: group A to be treated with 1% citric acid saturated with respect to hydroxyapatite (pH 3.6), group B to be treated with 1% citric acid without hydroxyapatite. The respective citric acid treatments were for 4 hours, rinsed with distilled water, and digital images were taken again. ImageJ was used to measure the RGB (red, green, blue) values. These values were statistically compared using a paired t-test.

Essential results: For group A, the stain was significantly reduced by 18.9 +/-8.7%, $p<0.0001$ (paired t-test). For group B, the stain was not significantly reduced, 0.93 +/- 12.9%, $p=0.6376$ (paired t-test).

Conclusion: This data confirms our hypothesis that citric acid saturated with respect to hydroxyapatite significantly reduces the stain caused by SDF treatment. Our previous erosion study found that hydroxyapatite saturated citric acid did not result in tooth mineral loss (Meola, 2023). Therefore, this method for stain reduction is effective in both stain reduction and prevention of erosion.

Title: Assessing the Mechanism of *Streptococcus* Inhibition by Azobenzene Copolymers

Authors: Nisali Piyasena, David Danforth, Joy Foster, Devatha Nair, and Michael Schurr

Category: Dental students and ISP students at the School of Dental Medicine

Background:
Acrylated hydroxyazobenzene (AHA) copolymers are inhibitory to *Streptococcus mutans*, contributing to dental caries' development. Dental caries has an overwhelming global presence, impacting over 2 billion people. This study aims to understand the mechanism of inhibition of AHA copolymers towards *Streptococci*. This knowledge will further the progression toward utilizing AHA copolymers in clinical settings.

Methods:
Samples were made using 3 mL of brain-heart infusion (BHI), dialyzed brain-heart infusion (dBHI), dBHI with 12.5 μM FeCl3 and 100 μL of 10^5 CFU/mL *S. mutans* in phosphate-buffered saline (PBS) with BisGMA:TEGDMA samples with/ without 2 wt% AHA copolymer). The samples were incubated (37°C, 5% CO2) for 6 hours. 10 μL were extracted hourly during incubation and serially diluted using PBS. Bacterial growth was analyzed using the Colony-Forming Unit Assay and the Geometric Viability Assay. For GVA, samples were combined with agarose (0.66% w/v solution in BHI) and triphenyl tetrazolium chloride (25 mg/mL 1000x in H2O). Samples were then suspended in micropipette tips (p200), incubated (37°C, 5% CO2) for 24 hours, and imaged using a mirrorless camera. Studies were repeated using ZnCl2 and MgCl2.

Results:
Samples with AHA completely inhibit *S. mutans* only in the presence of FeCl3. Samples containing ZnCl2 and MgCl2 did not exhibit S. *mutans* inhibition.

Conclusions:
AHA copolymer-based inhibition of *S. mutans* relies on the presence of iron, indicating that the Fenton reaction contributes to the AHA inhibition of *Streptococci*. Future tests will include studies that focus on mass spectrometry to analyze the specific iron-AHA interaction that generates the inhibitory effect.
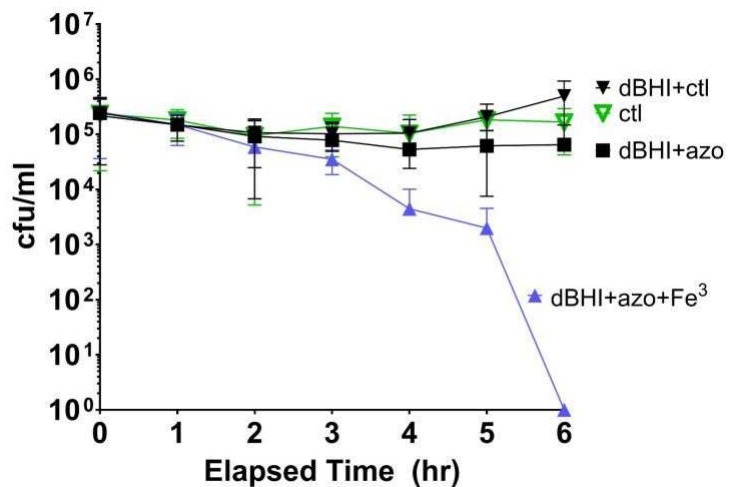
Figure 1 *Kill curves demonstrating AHA inhibition of S. mutans with/without Fe^{3+} presence.*

Title: A Photographic Method to Estimate Shade Change Due to Bleaching

Author: Edwin Sarkisians and Clifton Carey

Category: Postdoctoral Fellow/Resident

Tooth bleaching has become popular, but there is still no reliable method to accurately measure shade changes. The most common approach currently involves matching shade guides to the teeth before and after bleaching, which is highly subjective. This method depends on factors such as ambient lighting, the observer's visual acuity, and the condition of the teeth's surroundings, making it inconsistent.

Objective: To create a reproductive method and assess an in vitro photographic technique for measuring shade changes resulting from bleaching.

Experimental method: A Sony Alpha NEX-5N camera was used with 1/5 shutter speed, F3.5 aperture, and ISO 100. A polarizing filter was positioned at a 90-degree angle to the light source's polarization, creating a cross-polarized setup. The lighting system used dual 60 Hz LED lights, each with 0-degree linear polarizing filters and light diffusers. A dark box was employed to prevent reflections and maintain consistent lighting conditions. Six samples were matched to a shade guide, bleached for 1 hour in 10% Hydrogen Peroxide, rinsed with distilled water, and photographed. The samples were then soaked in distilled water for 7 days and rephotographed. Blue intensity in the RGB images was measured using ImageJ software.

Essential results: The blue intensity for each stage is shown in the graph. The paired t-test comparing the Blue intensity before and after bleaching is significant at $p=0.0092$; where the comparison after bleaching and at 7 days is not significant at $p=0.0764$.

Conclusion: The developed photographic method provided consistent and objective results for measuring shade changes due to bleaching. Significant differences were observed between pre-bleaching and post-bleaching stages
($p=0.0092$), and changes between post-bleaching and 7 days later were not significant
($p=0.0764$). This technique offers a reliable, reproducible approach for assessing bleaching effects through quantifiable data.
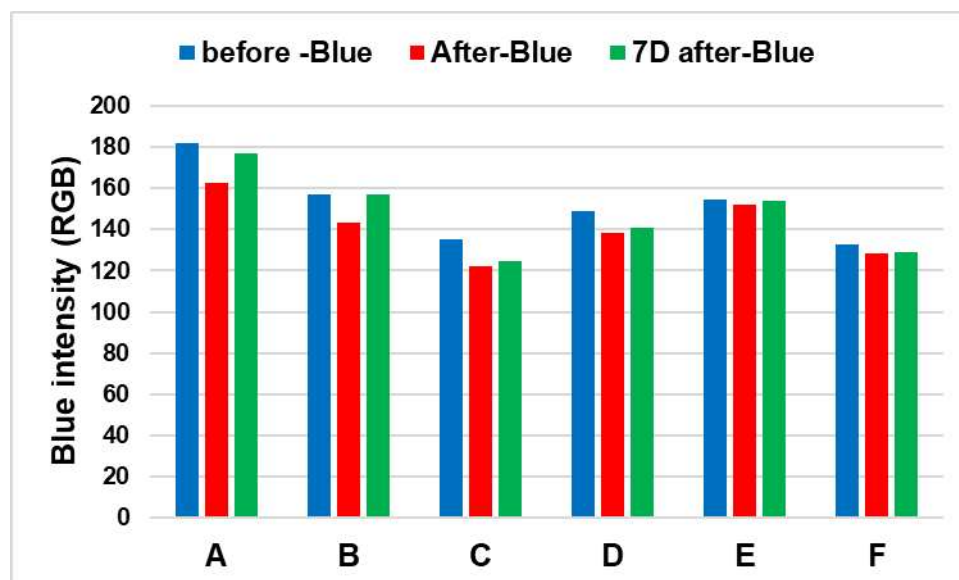
Title: Dental Students' Perceptions on International Service-Learning Activities at a US and Brazilian dental school

Authors: Malgorzata Waz, Larissa H. Morigaki, Samia P. Soares, Enzo E. Okazaki, Elizabeth Shick, Raquel Baroni de Carvalho, Bruce A. Dye

Category: Dental students and ISP students at the School of Dental Medicine

Institutions: (1) University of Colorado School of Dental Medicine; (2) Universidade Federal do Espírito Santo School of Dentistry

Objectives: Short-term international service trips (STISTs) are common in health professional programs. Recently, concerns about the purpose, mechanism of engagement, and the ethics of these trips have been articulated with a goal of ensuring activities should not take advantage of vulnerable populations or be disrespectful of local cultures and professionals. The objective was to assess dental students' perceptions on STISTs from two countries.

Methods: In 2024, a cross-sectional survey of dental students enrolled in the University of Colorado School of Dental Medicine (CUSDM) and the School of Dentistry, Federal University of Espírito Santo (UFES) was administered online, in English and Portuguese using Qualtrics. The survey instrument was comprised of 46 questions in 6 domains: Demographics and Experiences, Didactic Learning, Clinical learning, Community-Based Learning, Communication Perspectives, and Alignment with Ethical Guidelines.

Results: 147 students completed the survey (31% from CUSDM and 69% from UFES). Both groups preferred courses conducted in-person, desired to participate in a global health course, and had views aligned with modern ethical principles. It was less common for UFES students to have prior volunteering experiences ($p<0.0002$). Brazilian students were significantly more willing to participate in public health research activities on STISTs ($p<0.0012$) and strongly agreed that language proficiency is necessary to provide dental care or public health activities on STISTs ($p<0.001$). US students were more impacted by the availability of local language translator to determine types of community and public health activities ($p<0.00003$) or types of dental care to provide ($p<0.0003$) to provide.

Conclusion: Our findings showed some differences and similarities between American and Brazilian dental students regarding their preferences in global health experiences and education. These findings can help inform global health curricula development that incorporates STISTs address dental student interests while promoting community respect, autonomy, and beneficence since contemporary information in the literature is very limited.

Graduate
Student
Abstracts

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

Title: Characterizing the role of Akt-mediated phosphorylation of Srsf3 during mouse craniofacial development

Authors: <u>Charles W. Griffin</u>[1], Thomas E. Forman[1], Katherine A. Fantauzzo[1,2]

[1]Department of Craniofacial Biology, School of Dental Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO; [2]RNA Bioscience Initiative, University of Colorado Anschutz Medical Campus, Aurora, CO

Category: Graduate Student

Introduction/Background: Signaling through the platelet-derived growth factor receptor alpha (PDGFRa) is critical for craniofacial development in humans and mice. Mutations in *PDGFRA* are associated with cleft lip/palate in humans and *Pdgfra* mutant mouse models similarly display midline facial clefting phenotypes. Phosphatidylinositol 3-kinase (PI3K) is the primary effector of PDGFRa signaling during skeletal development in the mouse, leading to the activation of the serine/threonine kinase Akt. We previously showed 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 cells, resulting in its nuclear translocation. We further demonstrated that ablation of *Srsf3* in the mouse neural crest cell (NCC) lineage leads to severe midline facial clefting due to defective cranial NCC proliferation and survival.

Methods: Here we generated an *Srsf3* phosphomutant knock-in allele (*Srsf3*^{A7}) by replacing the terminal serine residue in the seven Akt consensus motifs in Srsf3 with an alanine residue using CRISPR/Cas9-based genome editing.

Outcomes/Results: We have not recovered any *Srsf3*$^{A7/A7}$ embryos from E8.5 through birth. To circumvent this embryonic lethality, we generated transheterozygous *Srsf3*$^{A7/fl}$;*Wnt1-Cre*$^{+/Tg}$ embryos in which the *Srsf3*^{A7} allele is the only *Srsf3* allele expressed in the NCC lineage. These embryos phenocopied *Srsf3* NCC conditional knock-out embryos, with facial process hypoplasia and severe midline facial clefting at mid-gestation. Further, introduction of the *ROSA26*^{mTmG} allele to examine NCC distribution revealed reduced GFP intensity in the facial processes of transheterozygous embryos at E9.5 and E10.5.

Conclusion: Phosphorylation of Srsf3 is necessary for proper craniofacial development, highlighting the first post-translational modification of an RNA-binding protein required in this setting.

Title: Investigating a new buffering mechanism for overcoming deleterious mutations

Authors: Grace Gustafson, Raisa Bailón-Zambrano, Abigail Mumme-Monheit, Juliana Sucharov, and James T. Nichols

Category: Graduate Student

Deleterious mutations can display a range of severity between individuals; in some organisms, mutations can be overcome through molecular mechanisms. This phenomenon of buffering against mutations has been accepted as a biological concept for decades. Individuals with the same mutation may vary in severity, assumedly due to their own unique genetic backgrounds. Transcriptional adaptation (TA) through nonsense- mediated decay has recently become a growing topic of study due to the molecular mechanism of compensatory paralog upregulation to protect against premature termination codons introduced in a single gene. This mechanism, however, is confined to nonsense mutations; mutations of other character exist that do not undergo TA, but do present less severe phenotypes than expected. Here we show one such allele with a mutation in the initiating methionine that was predicted to produce no protein, yet it unexpectedly produces a very mild phenotype, and we propose a novel mechanism of buffering that this allele undergoes. We discovered not only that this initiating AUG to CUG mutation produces protein through an immunofluorescence stain, but it also displays higher relative mRNA abundance than wild-type siblings, either due to increased stability or higher expression levels. We also identified the presence of in-frame downstream AUGs, that could provide alternative start sites for translation of a protein if it cannot initiate off of a CUG. Buffering has historically been understood only by characterizing phenotypic severity, however we now are able to investigate mechanisms on a molecular level. This novel mechanism is likely applicable across biological systems with similar mutations.

Project Title: Investigating the developmental origins of median fins through mutant analysis

Authors/Investigators: Margaret Keating, Raisa Bailon-Zambrano, Raelyn Begay, Grace Gustafson, Lindsey Barske, and James T. Nichols

Category: Graduate Student

Introduction/Background:
Paired appendages, like pectoral and pelvic fins, have been of great scientific interest due to their homology to tetrapod limbs. In contrast, the developmental origins of median appendages - dorsal, anal, and caudal fins - are often ignored, leaving large gaps in knowledge. However, the fossil record indicates that median fins evolved prior to paired fins. Furthermore, it is believed that the developmental programs for median fins were co- opted to build paired fins. At the center of this study are zebrafish mutant lines with striking, and opposing, median fin phenotypes. The *smb* and *eomesa* mutants have median fin reduction while the *pax9* mutant has an expansion. These mutants allow us to delve deeply into ancestral median fin development informing a fundamental mechanism of vertebrate appendage development from a largely unexplored angle.

Methods:
The mesenchymal population of dorsal and anal fin buds, transient structures akin to limb buds, were visualized by confocal imaging of *fli:EGFP*. The skeletons of these median fins were analyzed after staining bone with Alizarin Red and cartilage with Alcian Blue. Double mutant analysis was performed with the fin reduction mutants (*smb* and *eomesa*) and the gain of fin mutant (*pax9*).

Outcomes/Results:
The fin reduction mutants (*smb* and *eomesa*) did not have mesenchymal fin bud population of the dorsal fin and had a reduction in anal fin bud population. Contrastingly, the gain of fin mutant (*pax9*) had an expansion of the anal fin bud. Additionally, a loss of one copy of *pax9* rescued both the *smb* and *eomesa* phenotype.

Conclusion:
Both the *smb* insertion and *eomesa* act upstream of *pax9*, a negative regulator of fin bud population.

Title: Alternative RNA splicing of transcripts encoding protein serine/threonine kinases downstream of PDGFR signaling in the facial mesenchyme

Authors: <u>Cassandra B. Minne</u>[1], Brenna J.C. Dennison[1], Eric D. Larson[2,3], Katherine A. Fantauzzo[1,4]

[1]Department of Craniofacial Biology, School of Dental Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO; [2]Department of Otolaryngology – Head and Neck Surgery, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO; [3]Basic and Translational Sciences, Penn Dental Medicine, University of Pennsylvania, Philadelphia, PA; [4]RNA Bioscience Initiative, University of Colorado Anschutz Medical Campus, Aurora, CO

Category: Graduate student

Introduction/Background:
Craniofacial development is a complex morphogenetic process, disruptions in which result in highly prevalent human birth differences. Signaling through the platelet-derived growth factor receptors (PDGFRs) plays critical roles in this process in humans and mice. However, the gene expression changes that mediate cellular activity downstream of PDGFRα and/or PDGFRβ are incompletely understood.

Methods:
Here we performed sequencing of maxillary process mesenchyme RNA from E11.5 mouse embryos that lack *Pdgfra*, *Pdgfrb* or both in the neural crest lineage to examine the transcriptional output in each context.

Outcomes/Results:
DESeq2 analysis identified 23, 20 and 25 genes that were differentially expressed between *Pdgfra$^{fl/fl}$;Wnt1-Cre$^{+/Tg}$*, *Pdgfrb$^{fl/fl}$;Wnt1-Cre$^{+/Tg}$* and *Pdgfra$^{fl/fl}$;Pdgfrb$^{fl/fl}$;Wnt1-Cre$^{+/Tg}$* samples as compared to wild-type, respectively. In contrast, rMATS analysis detected over 5,000 differential alternative RNA splicing (AS) events per genotype compared to wild-type samples, with the majority of events involving skipped exons. Gene ontology analysis of the genes encoding the transcripts in the skipped exon category of each genotype revealed an enrichment for protein serine/threonine kinase activity functioning within the PI3K and/or MAPK signaling pathways. For approximately one third of these events unique to a single genotype, the same transcript was subject to AS in one or more of the remaining genotypes at a different exon.

Conclusion:
AS is the predominant mechanism of gene expression regulation downstream of PDGFR signaling in the facial mesenchyme. Further, signaling downstream of the various PDGFR dimers targets an overlapping set of transcripts encoding protein serine/threonine kinases for AS.

Title: Variable paralog expression underlies incomplete penetrance

Authors: Abigail Mumme-Monheit, Colette Dolby, Faith Frasier, Juliana Sucharov, James T. Nichols

Category: Graduate Student

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 are elusive. Using a zebrafish model of the human craniofacial disease *MEF2C* Haploinsufficiency Syndrome, we 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 highly conserved zebrafish *mef2* paralogs that arose from whole genome duplications (WGDs). However, *mef2ca* is the only paralog known to have craniofacial functions. Surprisingly, when these paralogs were removed in addition to *mef2ca* loss, *mef2ca* mutant-associated phenotypes worsened. We further found that *mef2* paralogs are more highly expressed in the low-penetrance strain compared to the high-penetrance strain, and that there is variation in paralog expression in unselected wild types. These findings indicate that heritable variation in paralog expression underlies heritable incomplete penetrance. Therefore, in this work I seek to test the hypothesis that heritable changes in DNA methylation and paralog expression underlie incomplete penetrance. I have used selective breeding to drive high or low phenotype penetrance and will evaluate differences in the genome, transcriptome, and methylome that segregate with penetrance. I also functionally test whether *mef2* paralogs can compensate for *mef2ca* loss using zebrafish transgenic lines. Thus far, I have demonstrated that the most closely related paralog, *mef2cb* can compensate for *mef2ca* loss. This work combines bioinformatic techniques with genome editing to precisely identify causes of incomplete penetrance and test whether incomplete penetrance can be driven in a laboratory setting. These experiments will elucidate whether paralogs can functionally rescue gene loss, and whether paralog expression is the engine of selection when breeding for incomplete penetrance. These findings provide insight into previously obscure cases of incomplete penetrance observed in *MEF2C* Haploinsufficiency Syndrome and other human diseases.

References:

1.   Sadr, Soroush, et al. "Deep Learning for Detection of Periapical Radiolucent Lesions: A Systematic Review and Meta-Analysis of Diagnostic Test Accuracy." *Journal of Endodontics*, vol. 49, no. 3, 1 Mar. 2023, pp. 248-261.e3, www.sciencedirect.com/science/article/pii/S0099239922008457?via%3Dihub, https://doi.org/10.1016/j.joen.2022.12.007. Accessed 3 May 2023.

2. Kumar V, Gossett L, Blattner A, Iwasaki LR, Williams K, Nickel JC. Comparison between cone-beam computed tomography and intraoral digital radiography for assessment of tooth root lesions. Am J Orthod Dentofacial Orthop. 2011 Jun;139(6):e533-41. doi: 10.1016/j.ajodo.2010.11.018. PMID: 21640865.

Title: Assessing AI Performance in Detecting Periapical Lesions on Intraoral Images

Authors: T. Al Jorani, **J. Ortiz**, B. Crockett, J. Parsons

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

Abstract: This study evaluates the effectiveness of Overjet AI, an artificial intelligence tool, as a diagnostic aid for detecting periapical inflammation in intraoral images. Traditional diagnostic methods for periapical inflammation are susceptible to observer bias, which can be mitigated by employing an unbiased AI model, potentially enhancing diagnostic accuracy.

Introduction/Background: Artificial Intelligence (AI) integration aids in identifying Periapical Radiolucent Lesions (PRLs) and improving diagnostics for dental professionals. Conventional methods rely on subjective interpretation of Cone Beam Computed Tomography (CBCT) vs Intraoral Imaging (IO), each with its limitations. CBCT often overestimates defects, while IO underestimates them, causing reporting bias.

Methods: 250 maxillary arch CBCT scans were reviewed by a certified Oral and Maxillofacial Radiologist for periapical lesions in endodontically treated teeth. Corresponding intraoral images within one year were analyzed using Overjet AI. Key performance metrics (sensitivity, specificity, positive predictive value, negative predictive value) were calculated to evaluate the software's diagnostic accuracy.

Outcomes/Results: Out of the 250 reviewed CBCT scans, 47 endodontically treated teeth exhibited periapical inflammation, and 125 showed no inflammation. For analysis, 32 cases from each group were selected based on recent intraoral imaging. The statistical analysis revealed the following:

True Positive (TP): 16
True Negative (TN): 28
False Positive (FP): 4
False Negative (FN): 16
Sensitivity: 50%
Specificity: 88%
Positive Predictive Value (PPV): 80%
Negative Predictive Value (NPV): 64%

Conclusions: AI showed 88% specificity in confirming the absence of periapical inflammation, with 50% sensitivity for its presence. Overjet AI reduces subjective bias and variability, complementing human observers. Integrating Overjet AI in dental diagnostics could improve reliability and consistency in assessing periapical inflammation.

IRB: #24-1684

Title: Identifying the subcellular compartment(s) that serves as the major signaling platform for PDGFR homodimers and heterodimers

Authors: <u>Gabriela M. Padilla</u>[1], Katherine A. Fantauzzo[1]

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

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

Introduction/Background: Craniofacial development is a complex morphogenetic process, disruptions in which result in prevalent human birth differences. Signaling through the platelet-derived growth factor receptor (PDGFR) family of receptor tyrosine kinases (RTKs) plays critical roles in this process in humans and mice. PDGFRα and PDGFRβ can homodimerize or heterodimerize in response to PDGF ligand binding, unleashing an intracellular signaling cascade. We recently adapted a bimolecular fluorescence complementation (BiFC) approach that enabled us to visualize and purify individual PDGFR dimers *in vitro*, revealing differences in the timing and extent of dimer activation, signal molecule binding, internalization, trafficking and downstream signaling. Moreover, our results supported an emerging theme in the RTK field that these receptors signal at sites other than the plasma membrane and require internalization to maximally activate downstream signaling.

Methods:
where we will identify the subcellular compartment(s) that serves as the major signaling platform for the various PDGFR dimers using biologically-relevant primary mouse embryonic palatal mesenchyme (MEPM) cells derived from embryos in which the stop codon of the endogenous *Pdgfra* and/or *Pdgfrb* locus was replaced with a BiFC fragment(s).

Outcomes/Results: We have confirmed BiFC expression in the facial mesenchyme of PDGFR-BiFC embryos. Further, we have cultured primary MEPM cells from these embryos and confirmed colocalization of the BiFC signal with the relevant PDGFR(s) and phosphorylation of downstream signaling molecules Akt and Erk1/2 upon stimulation of these cells with the relevant PDGF ligand. Finally, we have optimized transfection conditions to introduce plasmids encoding wild-type and dominant-negative Rab GTPases which mediate trafficking to particular endosomal compartments into immortalized MEPM cells.

Conclusion: PDGFR-BiFC embryos and cultured MEPM cells are a powerful tool to probe the mechanisms underlying the spatial and temporal regulation of RTK signaling during mammalian craniofacial development.

Title: *alx* function in the frontonasal skeleton revives the pharyngeal arch-0 hypothesis.

Authors: Nadia Wright, Jennyfer M. Mitchell, Colette Dolby, Matthew Murry, James T. Nichols
Category: Graduate Students in labs at the School of Dental Medicine

Segmentation is a universal organizing principle among bilaterian animals and divides the embryo into a series of repeating units that give rise to specific structures during development. Evolution acts upon these repeating segments to make them different. In contrast, the reverse process of homeotic transformation makes them the same. The vertebrate head arises from cranial neural crest cells in the segmental series of pharyngeal arches, patterned by nested homeobox genes that display homeotic transformations when mutated. There are cranial neural crest cells residing anterior to the pharyngeal arches that give rise to the midface, but these are not considered arches because they do not express arch markers. Conversely, the *alx* transcription factor encoding gene family is strongly and specifically expressed in frontonasal neural crest cells. Here we show that *alx* mutants have striking craniofacial phenotypes and propose that the anteriormost neural crest cells are segmentally homologous with the pharyngeal arches through *alx* mutants. We discovered that *alx1;alx3;alx4a* triple mutants appear to have duplicated upper jaw structures, which arise from pharyngeal arch 1, that form in the place of the midface. We found that in mutants, cells are present in the frontonasal region like in wild types but have expanded ectopic expression of pharyngeal arch 1 markers when compared with wild types. Moreover, single-cell RNA-seq of *alx3;alx4a* mutants suggests that there is a gain of pharyngeal arch identity at the expense of frontonasal identity in double mutants. Our findings demonstrate that *alx* confers positional identity within the frontonasal population. These *alx* mutations results in a gain of pharyngeal arch identity at the expense of frontonasal identity, indicating a homeotic transformation. This motivates a reexamination of the classical evolutionary theory that speculated that there was a pharyngeal arch segment anterior to pharyngeal arch 1.

Postdoctoral Fellows & Resident Abstracts

School of Dental Medicine

UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

Title: A Closer Inspection at the Vertebrate Head Reveals Ancestral Patterning Domains

Authors: Raisa Bailon-Zambrano, Jennyfer M. Mitchell, Nadia Wright, Daniel Medeiros, James T. Nichols

Category: Post Docs and Residents in labs at the School of Dental Medicine

In zebrafish larvae, the frontonasal skeleton, or anterior neurocranium, consists of the ethmoid plate and trabecular cartilages, and the parasphenoid bone. We reported that the *alx* gene family of transcription factors is enriched in frontonasal neural crest cells (fNCC) which reside anterior to the pharyngeal arch one. Functional genetic analyses of this gene family suggest they confer spatial identity in the developing anterior neurocranium; combinatorial mutants of alx genes present cartilage that resembles jaw structures in the place of frontonasal skeletal elements. The expression of dlx genes is restricted to the pharyngeal arches which give rise to the viscerocranium, including the jaw. Consistently, dlx genes are ectopically expressed in fNCC in these mutants.
Previous work on the basal vertebrate, lamprey, concluded that orthologs of these genes overlap in the frontonasal domain of the developing lamprey head suggesting this patterning might have arisen in more recent vertebrates. However, these conclusions were drawn from in situ analyses that did not allow for proper spatial information along the medial-lateral axis and probing of multiple genes in the same sample. Using modern techniques such as Hybridization Chain Reaction and confocal microscopy, we re- examined the spatial domains of these genes in the lamprey developing head and how they compare to the zebrafish head domains. We found that similar to zebrafish, Alx expression in lamprey is restricted to the medial region of the frontonasal domain, is anterior to, and does not overlap with any Dlx expression at early embryonic stages. These results suggest an ancestral genetic circuitry and domains already present in stem vertebrates. Altogether, this data motivate a reexamination of the classical, controversial "arch zero" hypothesis where the vertebrate neurocranium is derived from an ancestral homologous pharyngeal segment residing anterior to arch one. These findings raise fundamental questions regarding how this subpopulation contributes to vertebrates' frontonasal development.

Title: Pain, efficacy and preference associated with needle-free anesthetic injections compared to needle injections for orthodontic TAD placement: a prospective randomized clinical trial.

Authors: Bracken R. Bateman, Clifton Carey, Gerald Minick, W. Craig Shellhart

Category: Post Docs and Residents in labs at the School of Dental Medicine

Introduction: TAD placement is on the rise in orthodontics but pain and fear associated with needle injection is still present. The purpose of this study was to evaluate patients' pain and preference upon intraoral needle-free injected anesthesia versus conventional needle injected anesthesia for orthodontic temporary anchorage device placement evaluated both upon anesthetic placement and TAD placement.

Materials and Methods: Seventeen orthodontic patients were assessed for eligibility and 14 requiring bilateral TADs were enrolled, 2 dropped out for a total of 12 that completed the study. Allocation was randomized by anesthetic delivery method, side of the mouth, and order of delivery. Each patient received needle-free injection on one side of the mouth and needle injection on the other. Pain was measured on a 100-mm visual analog scale (VAS) prior to procedure, after anesthetic delivery, and after TAD placement and compared between the two anesthetic delivery methods. Following the procedure, the patients were given a questionnaire asking which delivery method they preferred.

Results: There was no statistical difference in pain during anesthetic delivery (P= 0.8789) or during TAD placement (P=0.5098) with either needle-free or needle injection delivery method. There was no statistical difference in preference between the two methods.

Conclusions: At this point, neither needle-free anesthetic nor needle injected anesthetic method was significantly more painful during placement or TAD insertion. No patient preference exists between the two techniques. The power of the study is lacking and indicates the need for more participants.

Title: Differential Srsf3 protein interactions upon PDGFRα signaling in mouse embryonic palatal mesenchyme

Authors: <u>Evan C. Brooks</u>[1], Thomas E. Forman[1], Katherine A. Fantauzzo[1,2]

[1]Department of Craniofacial Biology, School of Dental Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO; [2]RNA Bioscience Initiative, University of Colorado Anschutz Medical Campus, Aurora, CO

Category: Post Docs and Residents in labs at the School of Dental Medicine

Introduction/Background:
Craniofacial development is a complex morphogenetic process, disruptions in which result in highly prevalent human birth differences. Signaling through the receptor tyrosine kinase (RTK) platelet-derived growth factor receptor alpha (PDGFRα) plays critical roles in this process in humans and mice. We previously identified the RNA-binding protein (RBP) Srsf3 as an effector of PDGFRa signaling that regulates alternative RNA splicing following phosphorylation-dependent translocation into the nucleus.

Methods:
To identify proteins that differentially interact with Srsf3 depending on its phosphorylation in response to PDGF-AA ligand stimulation, immortalized mouse embryonic palatal mesenchyme (iMEPM) cells were treated with PDGF-AA ligand for 0, 15 and 60 min. Srsf3-interacting proteins were isolated via immunoprecipitation and analyzed by mass spectrometry.

Outcomes/Results:
Our screen identified 52 unique proteins, 20 and 32 of which had increased and decreased spectral counts upon PDGF-AA ligand treatment, respectively. These proteins included 17 that have previously been implicated in RNA binding. Consistent with the mass spectrometry results, we have biochemically confirmed the increased interaction of Srsf3 with one of these proteins implicated in a human craniofacial syndrome upon PDGF-AA ligand stimulation of iMEPM cells via immunoprecipitation and western blotting.

Conclusion:
Phosphorylation of Srsf3 downstream of PDGFRα signaling leads to differential interaction with other RBPs. Future studies will focus on whether these protein-protein interactions require RNA and affect Srsf3 subcellular localization and/or Srsf3-mediated alternative RNA splicing.

Title: PDGFRa/b heterodimer activation negatively affects downstream ERK1/2 signaling and cellular proliferation

Authors: Maria B. Campaña[1], Madison R. Perkins[1], Maxwell C. McCabe[2], Andrew Neumann[1], Eric D. Larson[3], Katherine A. Fantauzzo[1*]

[1]Department of Craniofacial Biology, School of Dental Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO; [2]Department of Biochemistry and Molecular Genetics, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO; [3]Basic and Translational Sciences, Penn Dental Medicine, University of Pennsylvania, Philadelphia, PA

Category: Post Docs and Residents in labs at the School of Dental Medicine

Introduction/Background:
The platelet-derived growth factor receptor (PDGFR) family of receptor tyrosine kinases allows cells to communicate with one another by binding to growth factors at the plasma membrane and activating intracellular signaling pathways to elicit responses such as migration, proliferation, survival and differentiation. 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. The PDGFR family consists of two receptors, PDGFRa and PDGFRb, that dimerize to form PDGFRa homodimers, PDGFRa/b heterodimers and PDGFRb homodimers.

Methods:
Here we tested the hypothesis that differential internalization and trafficking dynamics of the various PDGFR dimers underlie differences in downstream intracellular signaling and cellular behavior. We generated and analyzed a cell line stably expressing C-terminal fusions of PDGFRa and PDGFRb with bimolecular fluorescence complementation fragments of the Venus fluorescent protein. This approach allowed us to visualize and purify PDGFRa/b heterodimers for the first time.

Outcomes/Results:
We found that these receptors heterodimerize relatively quickly in response to PDGF-BB ligand treatment, with a peak of receptor autophosphorylation following 5 minutes of ligand stimulation. Moreover, we demonstrated that PDGFRa/b heterodimers are rapidly internalized into early endosomes, particularly signaling endosomes, where they dwell for extended lengths of time. We showed that PDGFRa/b heterodimer activation does not induce downstream phosphorylation of ERK1/2 and significantly inhibits cell proliferation. Further, we characterized the PDGFR dimer-specific interactome and identified MYO1D as a novel protein that preferentially binds PDGFRa/b heterodimers. We demonstrated that knockdown of MYO1D leads to retention of PDGFRa/b heterodimers at the plasma membrane, resulting in increased phosphorylation of ERK1/2 and increased cell proliferation.

Conclusion: Collectively, our findings impart valuable insight into the molecular mechanisms by which specificity is introduced downstream of PDGFR activation to differentially propagate signaling and generate distinct cellular responses.

Title: Investigating the role of intracellular Ca^{2+} dynamics in lower jaw development

Authors: Stanley M. Kanai, James T. Nichols, and David E. Clouthier

Category: Post Docs and Residents in labs at the School of Dental Medicine

Many tissues in development are patterned by morphogen gradients, a mechanism where differences in local morphogen concentrations instruct cells to adopt different positional identities. Patterning of the lower jaw requires the peptide ligand Endothelin-1 (Edn1) and Endothelin Receptor Type A, which elicit transient spikes in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) through Gq/11 and Phospholipase Cβ signaling effectors. Although a wealth of evidence suggests that an Edn1 morphogen gradient patterns the facial mesenchyme for lower jaw structures, the cranial neural crest cells (NCCs) in pharyngeal arch 1, this has never been formally tested. Here, we test the morphogen gradient model by quantifying spatial and temporal dynamics of $[Ca^{2+}]_i$ oscillations in live zebrafish embryos. The model predicts that $[Ca^{2+}]_i$ oscillations will exhibit higher amplitudes and frequencies in ventral cells adjacent the Edn1 source relative to more dorsal-positioned cells. $[Ca^{2+}]_i$ oscillations in NCCs were measured in double transgenic zebrafish [*Tg*(*sox10:Gal4;UAS:GCaMP7a*)] between 22-26 hours post fertilization using confocal microscopy. We show that $[Ca^{2+}]_i$ oscillations were reduced in cranial NCCs of *edn1*$^{-/-}$ embryos, whereas distinct patterns of $[Ca^{2+}]_i$ oscillations were observed along the dorsal-ventral axis in *edn1*$^{+/+}$ embryos. Specifically, the frequency and amplitude of $[Ca^{2+}]_i$ oscillations were highest ventrally and diminished dorsally. *edn1*$^{+/-}$ embryos exhibited similar patterns, but the overall frequency and amplitude was lower compared with *edn1*$^{+/+}$ embryos – a surprising discrepancy given *edn1*$^{+/-}$ embryos never exhibit craniofacial phenotypes. Our results thus far support the Edn1 morphogen gradient model and reveal an unexpected relationship between Ca^{2+} signaling and *edn1* gene dosage.

Title: Pain control in orthodontic patients using combined acetaminophen and ibuprofen compared to ibuprofen alone or placebo: a single-center, blinded, randomized, controlled, clinical trial.

Authors: Morgan Lane*, Clifton Carey, Gerald Minick, Craig Shellhart

Category: Post Docs and Residents in labs at the School of Dental Medicine

Introduction:
Pain is an inevitable effect of orthodontic treatment. Common over the counter analgesics are ibuprofen and acetaminophen. These two drugs do not always provide adequate pain control for patients. A combination of acetaminophen and ibuprofen has been found to provide more effective pain control in oral surgery and endodontics; however, its efficacy has not been reported in orthodontics.

Materials and Methods:
This clinical trial was triple-blinded. Each participant was provided a blister pack of capsules and were instructed to take three capsules at time points 0, 6, 12, 24, 48, 72, and 96 hours after placement of separators or initial arch wire. The participants filled out a visual analog scale (VAS) at each time point to record their pain level. Outcome measures evaluated were maximum pain and total pain reported (area under the curve) over the 4-day period.

Results:
The combination ibuprofen and acetaminophen group reported the lowest peak pain (30.6 ±26.6, 12h) followed by ibuprofen (33.33±22.98, 48h) and then placebo (36.25 ±24.10, 12h) with no statistical significance (p>0.05) at any time point. The total pain reported ranked with the combination group experiencing the least amount of pain followed by ibuprofen and then placebo (1622.8, 2389.7, 2495.1 VAS-hours), respectively, with significance at p=0.0002.

Conclusions:
No definitive conclusions can be made at each time point because the study is ongoing and requires more participants and data to achieve sufficient power. The current data indicates that combination ibuprofen with acetaminophen yields better total pain reduction than ibuprofen alone or placebo.

Title: Comparison of self-reported pain associated with initial placement of conventional, superelastic, or thermoelastic nickel-titanium archwires: a blinded, controlled, randomized clinical trial.

Authors: Elaine Lee*, Clifton Carey, Gerald Minick, Craig Shellhart

Category: Post Docs and Residents in labs at the School of Dental Medicine

Introduction:
Patients often experience pain associated with early orthodontic wires placed during fixed appliance therapy. The perceived pain can be debilitating enough to hinder daily functions like chewing, decrease compliance, and lead to the desire to discontinue orthodontic treatment. Variation in force levels produced by different types of nickel-titanium archwires may result in different amounts of pain experienced by patients. The purpose of this study was to compare the level of pain at specific time points after initial archwire placement experienced by orthodontic patients who received one of three types of nickel-titanium archwires: conventional NiTi, superelastic NiTi, and thermoelastic NiTi.

Materials and Methods:
A total of 89 participants received either conventional, superelastic, or thermoelastic NiTi wires after fixed appliances were bonded. The level of pain was reported by responding to a digital VAS at 7 timepoints (baseline, immediately after archwire insertion, 1 hour, 4 hours, 24 hours, 4 days, and 7 days). Additionally, participants were asked to report any analgesic usage at each timepoint.

Results:
Participants who received the superelastic NiTi reported higher level of pain 1 hour after archwire insertion when the data from subjects who reported taking pain medication was included (p=0.0085) and higher level of pain at 1 hour (p=0.043) and 4 hours (p=0.044) after archwire insertion when data from subjects who reported taking pain medication was excluded.

Conclusions:
Superelastic NiTi archwires elicit greater pain than conventional and thermoelastic NiTi archwires 1 hour after insertion.

Title: Development of a method for age estimation for adults using a CBCT derived pulp/tooth area ratio method: a controlled retrospective, observational study.

Authors: Hanna Lee*, Clifton Carey, Gerald Minick, Craig Shellhart, Klint Butler

Category: Post Docs and Residents in labs at the School of Dental Medicine

Introduction:
Accurate age estimation methods are critical, especially for individuals lacking documentation, yet challenges remain—particularly in adults—due to completed root development. Secondary dentin formation and pulp-tooth area ratios are promising but require further study. This study evaluated the accuracy of age estimation using pulp-to-tooth area ratio in permanent dentition for individuals aged 18 years and older using Cone Beam Computed Tomography (CBCT).

Materials and Methods:
This retrospective study evaluated the accuracy of age estimation using pulp-tooth area ratios in patients before and after orthodontic treatment. A convenience sample of 16 patients (aged 15 to 70 years) was selected from the University of Colorado, School of Dental Medicine database. Chronological age was the independent variable, with measurements of tooth and pulp areas taken from pre- and post-orthodontic CBCT sagittal slices of upper canines. Data was analyzed using Dolphin Imaging and Image J software, and results were correlated with patient ages to assess the impact of orthodontic treatment on age estimation accuracy.

Results:
Preliminary pre-therapy analysis of CBCT scans from patients aged 15 and older revealed a negative slope with smaller pulp-tooth area ratios with increasing age. The data showed a significant correlation and a clear linear relationship (R=0.6825, p=0.0036), indicating consistent trends with age and pulp-tooth area ratios.

Conclusions:
This study is a work in progress with a promising preliminary model for adult age estimation.

Title: A comparison of orthodontic miniscrew stability and peri-implant inflammation in orthodontic patients when miniscrews are precoated with antimicrobial quaternary ammonium compound K21 or ethanol control on the surface: a triple-blind, split-mouth, randomized controlled clinical trial.

Authors: Sujeong Lee, Sophia Heitmiller*, Clifton Carey, Gerald Minick, Craig Shellhart

Category: Post Docs and Residents in labs at the School of Dental Medicine

Introduction:
Research indicates that orthodontic miniscrew failure rates and complications are strongly correlated to inflammation at the site of placement. One potential strategy to reduce inflammation is to coat the miniscrew with an antimicrobial quaternary ammonium silane solution (K21) to reduce failure rates of orthodontic miniscrews.

Materials and Methods:
Orthodontic patients who were treatment planned for miniscrew placement received identical miniscrew pairs (one treated with K21 and the other treated with placebo) following a standardized placement protocol. The identity of the miniscrew treatment solutions were labeled A or B to blind the investigators. Participants were monitored during two monthly follow-up visits. Stability was evaluated through gentle manipulation using cotton pliers, with clinical failure defined as mobility of ≥1 mm. Peri-implant inflammation was assessed using the Loe and Silness index.

Results:
A total of consented 20 participants have been enrolled in the study. During the 4-week follow-up, three failures occurred (2 in group A and 1 in group B), with no additional failures thereafter. At the 4-week follow-up, five participants (2 in group A, 3 in group B) exhibited inflammation around at least one miniscrew. At the 8-week follow-up, two participants in group B showed inflammation around at least one miniscrew. Two miniscrews in group B exhibited inflammation without failure, while all other miniscrews with inflammation were associated with failure. No adverse effects have been observed.

Conclusions:
Because this is an ongoing blinded study, no conclusions can be drawn about the effectiveness of K21 to reduce miniscrew failures.

Title: Use of toothpastes containing non-traditional ingredients including activated charcoal, nano-hydroxyapatite, potassium nitrate, calcium carbonate, coconut oil, 5% hydrogen peroxide, or without fluoride prior to bonding on orthodontic bracket bond strength in the shear mode; a controlled in vitro study.

Authors: Jereme Sebastian*, Clifton Carey, Gerald Minick, Craig Shellhart

Category: Post Docs and Residents in labs at the School of Dental Medicine

Introduction:

Patients are using a wide variety of toothpastes daily, some of which have effects on teeth that are not well understood. There are ingredients in these toothpastes that may be impacting the integrity of adhesives and bond strength of orthodontic brackets. The purpose of this study was to assess the effect of newer toothpastes on the bond strength in the shear mode (SBS) of orthodontic brackets.

Materials and Methods:

A total of 80 bovine teeth were collected, distributed into groups, and brushed with: 1000 ppm fluoride control, non-fluoride, activated charcoal, 5% hydrogen peroxide (H_2O_2), calcium carbonate ($CaCO_3$), potassium nitrate (KNO_3), nanohydroxyapatite (nHAP) toothpastes, and coconut oil containing slurries for a simulated 1-year brushing period. Teeth were rinsed and bonded with metal Twin brackets. After a 24-48 storage period, the SBS and adhesive remnant index scores (ARI) were recorded.

Results:

The $CaCO_3$ group resulted in an SBS below the recommended level of 5.9 MPa with an SBS of 5.5 MPa. The H_2O_2 (6.3 MPa) and nHAP (6.1 MPa) were both significantly lower than the control (10.8 MPa). The predominant mode of failure among all groups was within the adhesive at the tooth surface (ARI=1).

Conclusions:

From these in vitro experiments orthodontists should be aware that toothpastes containing nano-hydroxyapatite, calcium carbonate, and 5% hydrogen peroxide may decrease the SBS of bonded brackets.

Title: The effect of vibration therapy versus no vibration therapy on clear aligner sequential fit in orthodontic patients: a single center, prospective, randomized controlled clinical trial.

Authors: Colt Sicher*, Clifton Carey, Gerald Minick, Craig Shellhart

Category: Post Docs and Residents in labs at the School of Dental Medicine

Introduction:
Vibration technology accelerates bone remodeling in various medical treatments. This study aimed to evaluate its effect on tooth-aligner tracking during clear aligner therapy.

Materials and Methods:
Thirty subjects participated in an ongoing randomized controlled trial for clear aligner therapy. Participants were randomly assigned to a vibration therapy group or a control group. Aligners were scanned before treatment, and intraoral scans were taken after completing aligners. Images were superimposed, and point clouds measured discrepancies between the aligner's intaglio surface and intraoral scans.

Results:
Results found no significant difference in aligner fit between the AcceleDent and non-AcceleDent groups at any of the four time points. In the upper arch, the largest discrepancy occurred at week 16 (p=0.3825) and the least at week 8 (p=0.8311). For the lower arch, the largest discrepancy was at week 8 (p=0.2730) and the least at week 12 (p=0.8680). Overall, no significant differences were found between the upper and lower arches. T-tests and ANOVA revealed no significant differences between the control and AcceleDent groups in either arch.

Conclusions:
The data from this study indicated that the AcceleDent vibration therapy has no significant effect on the clear aligner sequential fit for orthodontic patients.

Title: Comparing 3D changes in volume of the nasal cavity via cone-beam computed tomography (CBCT) in patients undergoing maxillary skeletal expansion: a prospective clinical pilot trial.

Authors: Menaka Tandon*, Clifton Carey, Gerald Minick, Craig Shellhart, Benjamin Crockett

Category: Post Docs and Residents in labs at the School of Dental Medicine

Introduction:
Maxillary transverse deficiency can negatively impact a patient's quality of life by having an effect on eating, breathing, sleeping, and speech. In skeletally mature orthodontic patients, a MSE (Maxillary Skeletal Expander) is used to skeletally expand the maxilla without surgery, but little has been studied about the changes that occur in adjacent airway structures. The study is to determine the relationship between nasal cavity volume to the change in hard tissue landmarks or palatal expansion distance. The purpose of the pilot study is to establish the sample size needed to achieve 80% power at an α of 0.05.

Materials and Methods:
CBCT images were collected as part of the patient treatment plan. The CBCTs were collected at 4 time points: pre-expansion (T0), post-expansion (with MSE in place) (T1), after MSE is removed (T2), and after treatment is complete (T3). These are evaluated for palatal suture expansion, nasal cavity volume, and the expansion distance between the tips of the maxillary palatal cusps. The primary outcomes are the change in nasal cavity volume and maxillary expansion over time.

Results:
Preliminary results from 3 patients show that the change in nasal cavity volume from T0 to T1 was -0.8 ± 10.8% with a range of -10.3% to 10.9%.

Conclusions:
These data indicate that a sample size of 20 patients is needed to achieve 80% power at an α of 0.05 assuming a clinically significant change in nasal volume is 15%.

Title: Identification of WSLs in orthodontic patients with a novel nano-particle fluorescent caries detection system compared to visual detection: a controlled clinical trial.

Authors: Tahina Torres*, Clifton Carey, Gerald Minick, Craig Shellhart

Category: Post Docs and Residents in labs at the School of Dental Medicine

Introduction:
        Orthodontic patients are prone to developing white spot lesions (WSLs) due to the challenges of effectively removing plaque buildup around brackets. LumiCare Caries Detection Rinse™ is an innovative diagnostic solution that employs bioresorbable, starch- based nanoparticles tagged with luciferin, which penetrate the porous structure of WSLs. When exposed to blue light, these nanoparticles fluoresce, enabling the identification of WSLs. This pilot study aimed to compare the effectiveness of visual detection methods with LumiCare's fluorescence-based WSL identification.

Materials and Methods:
        Twenty patients who met the inclusion criteria participated in this pilot study. WSLs were initially identified visually, and location maps of the upper anterior teeth (canine to canine) were created for each patient by a single investigator. Following this, patients rinsed with the LumiCare disclosing solution for 30 seconds, followed by a 10-second water rinse. A second evaluator then created WSL maps using a blue LED curing light and orange safety glasses, which caused fluorescence of the starch-based nanoparticles absorbed by the lesions.

Results:
        Comparison of WSL maps of 20 participants (6 teeth x 4 quadrants/tooth x 20 participants = 480 surface areas) before and after the rinse found that there were 128 visually identified WSLs and 67 fluorescent WSLs identified. Thirty-two WSL were identified by both methods; 35 WSL were identified by fluorescence and not identified visually; 96 WSL were identified visually and not by fluorescence.

Conclusions:
        The results of this pilot study found that active WSLs are difficult to diagnose visually compared to non-active WSLs. More patients are needed in this study to establish the comparison of LumiCare fluorescent to visual WSL identification.

Title: A new model of cleft lip and palate demonstrates aberrant Notch signaling alters periderm formation and Wnt signaling dynamics

Authors/Investigators: Dianzheng Zhao, Erin Binne, Aftab Taiyab, Yunus Ozekin, Trevor Williams, Hong Li

Category: Post Docs and Residents in labs at the School of Dental Medicine

Introduction/Background: The Notch signaling pathway is highly conserved and regulates multiple developmental processes in a context dependent manner. Most studies on Notch signaling in orofacial clefting have been focused on the secondary palate because depletion of *Jag2*, a Notch ligand, causes cleft secondary palate. With respect to the lip and primary palate, our previous studies have shown that *Notch1* is the most abundantly expressed Notch ligand in the surface ectoderm of the facial prominences during development. Nevertheless, how modulation of Notch signaling in the facial ectoderm can impact the various aspects of face formation remains unclear. To fill this knowledge gap, we activated Notch signaling by overexpression of Notch1 intracellular domain in facial ectoderm (NotchGOF).

Methods: We analyzed the phenotypes of the NotchGOF mutants and further performed single cell RNA-seq (scRNA-seq) for the forming lip and primary palate regions derived from both control and mutant embryos.

Outcomes/Results: We observed significant disruptions in craniofacial morphology, including full penetrant bilateral cleft lip and palate, in the NotchGOF mutants. scRNA-seq analysis revealed context dependent roles of Notch signaling in the ectoderm. Thus, activated Notch promotes differentiation of ectodermal basal cells to periderm in surface ectoderm but inhibits differentiation of olfactory neuron cells in the nasal placode. Furthermore, both bioinformatic analysis and experimental validation demonstrated that Wnt signaling pathway crosstalk between ectoderm and mesenchyme, which is crucial for facial development, was attenuated in the NotchGOF mutants.

Conclusion: In summary, our study demonstrated that excessive Notch signaling can drive the differentiation of basal cells into periderm, alter critical Wnt signaling tissue interactions, and result in bilateral cleft lip and palate, revealing an unexpected role of Notch signaling in face development.

Research
Staff
Abstracts

CU | School of Dental Medicine

UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

Title: Characterization of the role of Srsf3 in the mouse facial ectoderm

Authors: Erin Binne[1], Brenna J.C. Dennison[1], Katherine A. Fantauzzo[1]
[1]Department of Craniofacial Biology, School of Dental Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO

Category: Research Staff at the University of Colorado School of Dental Medicine

Introduction/Background:
Craniofacial development is a complex morphogenetic process that requires a precise interplay of multiple cell types to generate the frontonasal skeleton. Defects in craniofacial development, including cleft lip/palate, comprise one of the most prevalent birth differences in humans. We have demonstrated that the RNA-binding protein Srsf3 is expressed in the mesenchyme of the pharyngeal arches and facial processes in mouse, as well as the overlying ectoderm, from E8.5 onwards. We showed that ablation of *Srsf3* in the mouse neural crest cell (NCC) lineage that contributes to the facial mesenchyme leads to severe midline facial clefting due to defective cranial NCC proliferation and survival.

Methods:
Here we sought to characterize a potential role for Srsf3 in the facial ectoderm. We combined an *Srsf3* conditional allele with the *Crect* transgene, which drives Cre recombinase activity in the cranial ectoderm beginning at E8.5. We assessed craniofacial phenotypes of control and *Srsf3* conditional knock-out (ect cKO) embryos through whole-mount DAPI staining, Ki67 immunofluorescence analysis and TUNEL staining of the frontonasal process (FNP), maxillary process (MxP) and mandibular process (MdP).

Outcomes/Results:
We found that *Srsf3* ect cKO embryos exhibit morphological defects by E10.5, including facial process hyperplasia and displaced nasal pits. Ki67 immunofluorescence analysis and TUNEL staining did not reveal any significant differences in proliferation nor cell death in the ectoderm nor mesenchyme between control and ect cKO embryos at E9.5. However, there were trends for increased Ki67 staining in the *Srsf3* ect cKO FNP and MdP, with decreased staining in the MxP. Additionally, there were trends for increased TUNEL staining in the *Srsf3* ect cKO FNP and MxP, with decreased staining in the MdP.

Conclusion:
Conditional ablation of *Srsf3* in the facial ectoderm results in facial process hyperplasia by E10.5, which does not appear to stem from cell proliferation nor survival defects.

Title: prrx1 genes regulate developmental timing of median fins

Authors: Colette Dolby, Raelyn Begay and James Nichols

Category: Research Staff Professionals working at the School of Dental Medicine

Limb development has been extensively studied in the context of normal development and human genetic diseases. Numerous studies have illuminated genes and processes required for the development of paired limbs in mice and zebrafish. It is widely hypothesized that paired fins arose from co-opting preexisting median fin pathways. It is surprising then that the more ancestral median fins have been severely understudied. Uncovering the machinery of median fin development may elucidate general principles of appendage formation and inform congenital limb abnormalities. Median fins arise from paraxial mesoderm, which condenses into fin bud mesenchyme. Previous studies have shown *prrx1a/b* expression in the mesenchyme of pectoral and pelvic limb buds and craniofacial skeleton. Skeletal stains of 3wpf larvae reveal abnormalities in the cartilage structures of all median fins in *prrx1a/b* mutants.

Our work supports the hypothesis *prrx1a/1b* regulate timing of developing median fin structures through inhibiting differentiation of progenitor stem cells until the appropriate time and place. Using a *prrx1a:RFP* transgene, we observed *prrx1a* expression in the developing trunk and median fins from 24 hpf persisting through 3 weeks post fertilization. Using *in situ* hybridization, we observed a greater volume of paraxial mesoderm stem cells in *prrx1a/1b* mutants compared to wild types at 24hpf. In *prrx1a/1b* mutants, we also observed an increase in caudal fin bud mesenchyme expressing high levels of the *fli1a:GFP* transgene at 6dpf. Our results are preliminary, but show when *prrx1a/1b* are mutated, then stem cell progenitors of median fins expand and differentiate precociously possibly resulting in the disorganization of later derived structures.

Title: A Look Inside Dental High-volume Evacuation Lines

Author: Christine Forrester

Category: Research Staff at the University of Colorado School of Dental Medicine

<u>**Objective**</u>:

To determine the effectiveness of our high-volume evacuation (HVE) lines and evacuation line cleaning products. Adequate aerosol removal requires an air flow rate of > 7 SCFM and ideally > 10 SCFM for proper aerosol mitigation.

<u>**Method:**</u>

Evacuation line flow was measured on three hundred and fifty-four (354) HVE lines using a flow meter. These lines were in service for 11 - 19 years and were cleaned weekly with Solution (A). The evacuation line flow was checked for each line at the J-box, suction container (without tubing attached) and at the HVE valve to determine a baseline flow for each unit.

Evacuation lines were randomly selected to be cleaned using solution B and a dispenser. The other lines were cleaned with Solution A (traditional solution) in the usual manner. The lines were checked after 2 - 10 months to determine airflow differences.

<u>**Results:**</u>

A total of 38% of the lines were below 10 SCFM with 5% below 7 SCFM, demonstrating that 43% of our evacuation lines were performing below an ideal range. The range and mean precleaning volume of airflow was 4.2 - 11.9 SCFM and 7.0 SCFM. Lines cleaned with Solution B and a dispenser showed a mean change of 1.26 SCFM in volume of airflow. There was no increase in airflow for lines cleaned with Solution A.

<u>**Conclusion:**</u>

Monitoring dental unit evacuation lines is an essential step in the maintenance of aerosol management. Over time the buildup of debris diminishes the effectiveness of the evacuation lines. Evacuation lines cleaned with Solution B showed continual improvement in volume of airflow capacity. This illustrates the impact of solution chemistry and method of dispensing a solution for evacuation line cleaning, has on improving and sustaining vacuum airflow for efficient aerosol mitigation during aerosol generating procedures.

Title: Properties of monourethane (meth)acrylates when used as low viscosity reactive diluents

Authors: Anna Gartner, Austyn Salazar, Jeff Stansbury

Category: Research Staff at the University of Colorado School of Dental Medicine

Objective: This study investigates monourethane (meth)acrylates used as alternative reactive diluents to enhance formulations for additive manufacturing applications by lowering viscosity while maintaining a high urethane concentration.

Methods: Monourethane (meth)acrylates were combined with high viscosity comonomers of urethane dimethacrylate (UDMA), and Ebecryl 270 (E270) with or without methacrylic acid (MAA). Formulations were made to ensure the viscosities were in the vat-printable range. All formulations, except those with E270, were made using a 1:1 molar ratio. Otherwise, they were made in a 10:1 comonomer:E270 ratio.
Viscosity was measured at 25°C and 250 rpm. Conversion was measured after four minutes of ambient cure and after 60 min/80 ºC post-cure processing. Samples of 2x2x25 mm were tested in 3-point bending to obtain mechanical properties.

Results: Monourethane-based formulations were created to produce photocurable resins with relatively low viscosities while delivering polymers with good mechanical properties by capitalizing on the extensive non-covalent bonding network present with the abundance of urethanes, either solely or augmented with added MAA. We focused on two monourethanes in particular, a monourethane dimethacrylate (MUDMA) and a polycaprolactone monourethane diacrylate (PCLMUDA). While MUDMA has stronger base mechanical properties than PCLMUDA, both monourethanes show a similar trend with noticeable improvements in modulus, flexural strength, and toughness with the addition of UDMA and MAA, both separately and together. This synergistic effect can be attributed to the reinforced noncovalent hydrogen bonding network between the urethane and acidic groups of the comonomers. The addition of the E270 also boosts the standard monourethane properties, however, not to the same extent as UDMA. E270 also presented the additional challenge of dropping the viscosity to an acceptable level, requiring greater amounts of the reactive diluents.

Formulation	Viscosity (mPa*s)	Postcure conversion (%)	Flexural Strength (MPa)	Flexural Modulus (GPa)	Flexural Toughness (MJ/m^3)
TEGDMA+UDMA	54.8±1.9	91.0±0.6	136.4±10.7	2.75±0.18	8.2±3.2
UDMA	8912±104	87.7±0.3	157.4±6.4	3.13±0.12	9.3±1.6
MUDMA	27.9±2.1	75.4±1.6	126.6±30.3	3.52±0.23	3.2±1.6
MUDMA+UDMA	461.8±38.9	85.5±0.5	147.9±13.3	3.33±0.11	5.9±2.6
MUDMA+MAA	13.9±1.0	87.7±1.1	163.8±28.3	4.24±0.25	4.5±2.0
MUDMA+UDMA+MAA	142.9±8.9	84.6±0.2	177.6±17.7	3.86±0.35	7.5±2.6
MUDMA+E270	614.3±24.1	86.7±1.0	118.3±4.6	2.38±0.15	9.9±3.1
MUDMA+E270 +MAA	132.5±9.4	89.6±0.3	186.4±8.7	3.91±0.16	10.0±2.7
PCLMUDA	65.2±5.8	100.0±0.0	9.3±0.7	0.13±0.04	1.4±0.3
PCLMUDA+UDMA	394.9±30.6	95.9±0.4	80.4±2.6	1.84±0.09	10.8±4.7
PCLMUDA+MAA	37.1±2.2	99.7±0.1	44.2±4.1	0.91±0.21	8.0±1.8
PCLMUDA+UDMA+MAA	17.7±3.4	95.2±0.1	183.3±5.7	3.61±0.22	16.6±5.0
PCLMUDA+E270	350.9±20.9	98.5±0.3	8.7±1.0	0.09±0.01	1.6±0.2
PCLMUDA+E270+MAA	90.7±7.8	96.2±0.3	57.4±3.5	1.13±0.11	9.9±1.2

Conclusion: This study has demonstrated that high urethane content photocurable resins are possible with the use of monourethane reactive diluents and acidic comonomers which maintain good properties while dropping the viscosity to an applicable level.

Title: Light-Propelled Azobenzene Nanogel for Dental Adhesive

Authors/Investigators: Sebastian Lopez, Humberto Escobedo, Jeffrey Stansbury, Devatha Nair

Category: Research Staff at the University of Colorado School of Dental Medicine

Introduction/Background:

Approximately 90% of adults suffer from tooth decay, fueling demand for resin-based restorations. However, current composite restoration lasts <10 years due to weak adhesive interface. By synthesizing light-propelled, cytocompatible methacrylated azobenzene nanogels (AB-NG) incorporated within traditional adhesives, we aim to develop mechanically robust adhesive layers creating long-lasting restorations.

Table 1. Molecular Weight and Size of synthesized AB-NG

Light-Responsive nanogels	Est Molecular Weight (kDa)	Size (nm)
AB-NG1	$2.14 \times 10^6 \pm 1.38 \times 10^6$	559.9 ± 136.76
AB-NG2	$1.86 \times 10^6 \pm 7.79 \times 10^5$	623.04 ± 272.75
AB-NG3	$9.99 \times 10^6 \pm 1.30 \times 10^7$	743.5 ± 680.37
AB-NG4	$4.94 \times 10^5 \pm 9.03 \times 10^5$	765.3 ± 656.31
AB-NG5	$3.78 \times 10^6 \pm 2.66 \times 10^6$	717.11 ± 251.05

Methods:

AB-NGs were synthesized from the monomers 2-(4-Hydroxyphenylazo) Benzoic Acid, Hexamethylene Diisocyanate, Glycerol, and 2-Isocyanatoethyl Methacrylate. The Zetasizer was used to estimate molecular weight and size of the AB-NG. To assess light-propelled movement, AB-NG (5-wt%) and the adhesive monomers bisphenol A-glycidyl methacrylate, Hydroxyethyl methacrylate, in 60:40 ratio, with 12-wt% ethanol were created. Directional diffusion was analyzed within capillary tubes, and the magnitude of photo-induced, directional flow effect (ΔS) was evaluated as $\Delta S = S_{final} - S_{initial}$. (**n**≥3). (Figure 1)

Outcomes/Results:

Molecular weight and size of synthesized AB-NG are shown in Table

1. The light-propulsion behavior of adhesives as a function of different types of AB-NG showed a diffusion length range of 2 mm to 23 mm.

Conclusion:

AB-NG within adhesive formulations undergo light-assisted directional diffusion, which will deliver adhesives to the depth of conditioning, enabling mechanically robust adhesive layers. Future studies will focus on AB-NG induced crosslinking between the tooth collagen and composites, ultimately increasing the longevity of restorations.
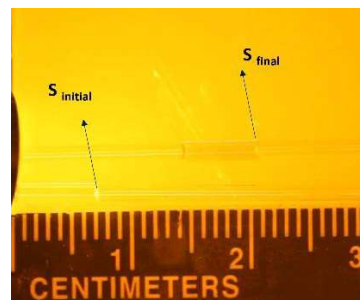
Figure 1. Light-propelled movement of AB-NG+ adhesive formulations measured in capillary tubes where $S_{initial}$ (before light exposure) and S_{final} (after light exposure) position of fluid was noted

Project Title: *POLR3A* is required for craniofacial cartilage and bone development

Authors/Investigators: Bailey Lubash, Roxana Gutierrez, and Kristin Watt

Category: Research Staff at the University of Colorado School of Dental Medicine

SEE POSTER PRESENTATION - VIEWING ONLY

Title: Formulating Photopolymer Systems for Multimaterial Jetting with a Range of Mechanical Properties

Authors: Austyn Salazar, Anna Gartner, Jeff Stansbury

Category: Research Staff Professionals working at the School of Dental Medicine

Objective: The purpose of this work is to highlight the spectrum of formulations ranging in modulus and strength intended for use in multimaterial inkjet 3D printing as applied to full and partial dentures.

Methods: Formulations were made to yield low modulus (<1 GPa) to high modulus (>4 GPa) photopolymers as an extension of previous work on urethane-based materials. A series of novel urethane (meth)acrylates were formulated with comonomers at different molar proportions to create homogeneous low viscosity inks that would increase modulus to a region that would benefit as a denture tooth material or lower the modulus to a region that would meet specific needs for denture base applications. 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: These formulations were made for use in multimaterial inkjet printing but minor adjustment in viscosity could be used in vat-based 3D printing as separate single-material parts for end-stage assembly. Denture base formulations require a modulus ~2.0 GPa which formulations **a-e** were intended ranging from 1-3 GPa. These formulations can also be mixed with higher modulus materials to tune properties to the target values. Formulation **a** and **e** are purposefully outside the 2 GPa modulus range for this reason. Formulations **f-m** are in the range of what denture tooth materials are currently peaking at, but the remaining formulations (**n-r**) were specifically designed to reach higher modulus and push the ceiling on the capability of denture tooth materials.
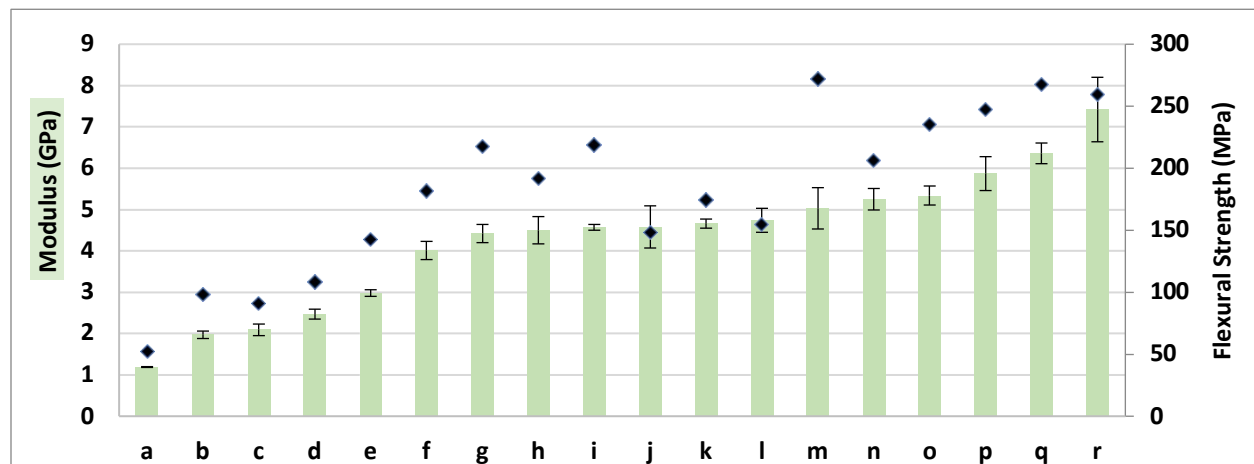
Conclusion: Formulating dental resins for the specific purpose of multimaterial printing has been successful due to the wide range of materials that have been designed and tested to tune modulus and strength based on the application. Denture base and denture tooth materials can both be printed either vat-based or with inkjet printing to attain tunable and personalized devices.

Faculty
Abstracts

School of Dental Medicine

UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

Title: Enamel damage after debonding monocrystalline ceramic brackets compared to debonding metal brackets in orthodontic patients a randomized control split mouth study.

Authors: Alejandro Arango*, Clifton Carey, Gerald Minick, Craig Shellhart

Category: Faculty at the University of Colorado School of Dental Medicine

Introduction:

The purpose of this study was to evaluate if debonding ceramic brackets will result in significantly more enamel surface roughness and damage depth than metal brackets before and after clean-up. Differences between the adhesive remnant index (ARI) scores for both brackets were also evaluated.

Methods:

The sample size calculation was based on a pilot study and previous research, estimating 10 samples per group for 80% power at alpha=0.05. Eight patients, three males and five females, undergoing comprehensive orthodontics with prescribed premolar extractions were recruited for this split mouth study. A total of twenty-six teeth were available for random allocation, thirteen in the metal bracket group and thirteen in the ceramic bracket group. Metal and ceramic orthodontic brackets were bonded with 3M Transbond-XT adhesive. Digital tooth surface images were collected before bonding (T0), after debonding one week later (T1), and after cleaning and polishing (T2). Enamel damage, including roughness, chips, cracks, and surface loss, was assessed using CloudCompare software.

Results:

Surface roughness measurements found no significant differences ($p > 0.05$) by paired t-tests between ceramic and metal brackets at debond (T1) and after adhesive cleanup (T2). However, significant reductions in surface roughness were observed from T1 to T2 across all samples (p=1.28E-06, power=100%). On average, surface roughness diminished substantially following adhesive cleanup, regardless of the bracket material.

Conclusions:

These findings suggest that the bracket material type does not impact surface roughness at T1, and the cleanup process effectively smoothens surfaces at T2.

Title: Empowering the Utility of Peer Teaching in Pre-clinical and Clinical Education

Authors: Yanira A. Owens, **Cori A. Hendricks**, *Armantine J.L. Lehn, Meghan E. Crow, and Michael Lampe

Category: Faculty at the University of Colorado School of Dental Medicine

Objectives: The peer teaching strategy has been utilized in health professions education including medical and dental education. While studies have examined the effectiveness of peer teaching in didactic coursework, there is limited data examining peer teaching in clinical dental education. This mixed methods study examined the value of peer teaching through a pilot peer instructor program (PIP) in a clinical dental education course.

Methods: Second-year dental students enrolled in the Periodontology Laboratory course and upperclassmen peer instructor dental students, selected and calibrated to peer teach in the Periodontology Laboratory course, were invited to participate in the study. Participants completed a cloud-based informed consent, a six-point Likert-scale survey, and one open-ended feedback prompt with a free-text response box. Statistical analysis included descriptive measures of frequencies, percentages, mean values, and Cronbach's alpha analysis. Qualitative data was analyzed with themes and coding.

Results: The response rate from the second-year dental students and peer instructors was 77% and 53%, respectively. Overall, second-year dental students (M = 4.10; SD = 1.03) and peer instructor dental students (M = 4.49; SD = 0.58) valued the PIP within their dental education. Qualitative results for second-year dental students included themes of *increase in clinical efficiency, unique clinical perspectives of peer instructors*, and *limitations of the PIP*. Themes from the peer instructors included s*trengthening of clinical foundational skills* and *a broad perceived educational value of the PIP.*

Conclusion: The results of the study demonstrated the peer teaching strategy, facilitated through the pilot PIP, is a valuable educational adjunct. The study's findings suggest that the PIP was successful in the delivery of peer teaching, and that the PIP should continue to be incorporated in clinical dental education.

Title: Increasing Student Engagement in General Pathology with Two Teaching Tools

Authors: Tanya Wright, DDS, Jennipher Murphy, MS-Ed, **Catherine Flaitz, DDS, MS***

Category: Faculty at the University of Colorado School of Dental Medicine

Abstract: General pathology provides a foundation for integration to clinical sciences by understanding systemic conditions which may occur in patients treated in student dental clinics. Complete Anatomy Application (Elsevier) allows students to apply knowledge obtained from gross anatomy and physiology courses through videos and 3-D models.

Objectives: This study incorporated the utilization of patient cases from the dental school clinics and Complete Anatomy to supplement the learning experience in the general pathology course and determine if these tools were useful.

Methods: IRB review set status as exempt. Eighty first-year dental students were asked a series of six self-reflection questions about the clinical cases and Complete Anatomy utilization and relevance to the course. Five questions applied the Likert 5-point scale ranging from strongly agree to strongly disagree and one free text for general comments. Student comments were broadly categorized by theme. Descriptive statistics were used for analysis.

Results: More than sixty students (80%) strongly agreed that the cases from the dental clinics made the discussion of learning the general pathology content more relevant while seven students (9%) felt the same utilizing Complete Anatomy. Twenty-four students (30%) did not use the app at all. The free text comments included positive feedback for the incorporation of clinical cases into the lecture material. Although a few students commented that the app had value, they cited time constraints/condensed course schedule as a deterrent for routine use.

Conclusion: General pathology concepts reinforced through incorporation of real-life case scenarios interspersed throughout the lecture material was well-received and improved learning. Some students felt the app could be useful as a resource to supplement the course material if time was allotted. Providing viable options to help reinforce biomedical science concepts and utilizing actual cases from the dental clinics is appreciated by the students and thereby provides a practical learning opportunity.

Title: Aged out and Lost! Establishing a Dental Home for Adults with Special Healthcare Needs

Authors: Susan Somserset, Abidin Tuncer, Katherine Chin, William Rivera, Bruce A Dye

Category: Faculty at the University of Colorado School of Dental Medicine

Issues: Patients with special healthcare needs (SHCN) because of significant disabilities face multiple hurdles transitioning from pediatric dental care. These include finding adult providers to provide services to adults (SHCN) and navigating health transitions without the integrated care teams seen in the pediatric care setting. Patients move into this new chapter as adults and are responsible for healthcare decisions. When patients lack decisional capacity, caregivers face the challenge of obtaining guardianship or medical proxy. Without a transition pathway, challenges seeking care quickly escalate, leading patients to withdrawing from receiving regular dental care.

Findings: Review of patient electronic dental record and current referral processes identified several areas for improvement for transitioning patients, even when the transfer is between "partnering" institutions. The initiating process was similar for all referrals and was significantly dependent on the patient/caregiver. This resulted in a small percentage of patients transitioning from child to adult dental care becoming a patient of record at the University Special Healthcare Needs Adult Clinic (SCC). The University Clinic implemented a new intake process for accepting patients (SHCN) age ≥ 16 that began with a clinic coordinator to navigate pre-appointment documentation, secure necessary records, and manage scheduling based on patient specific accommodations.

Conclusion(s): The implementation of the new intake process has increased patient retention, communication with the medical team and patient/caregivers and decreased missed appointments. This has resulted in a significant increase of eligible patients aging out of Children's Hospital to acquire a dental home at the University SSC. A dedicated clinic coordinator was a high impact change to caregiver/patient satisfaction and retention, while promoting oral health equity for those with disabilities. Finally, these care pathway processes can serve as a template for accepting referrals across a spectrum of pediatric dental care providers.

Data collection is ongoing and will be updated at poster presentation.
We need three learning objectives for your presentation to apply for educational credit consideration.
E
xample: "To understand the appropriate application of psychotherapy in patients with intellectual disability."

Learning Objective #1
To understand the barriers encountered for patients with special healthcare needs when transitioning from pediatric to adult providers.

Learning Objective #2
To understand the benefits of implementing a transition program for patients with special healthcare needs.

Learning Objective #3
To identify important documentation to request prior to the patient's appointment.

Multiple-choice questions with **four or more possible answers** are preferred. **Indicate the correct answer.**

Question/Answer(s) #1
What barriers are encountered by patients with special healthcare needs when transitioning from pediatric to adult dental providers?
A. Identifying providers that are able to care for patients with special healthcare needs
B. Having appropriate guardianship or medical decision making processes in place.
C. Communicating patient specific accommodations for successful dental care
D. All answers are correct (CORRECT ANSWER)

Question/Answer(s) #2
Patients with a court appointed guardian can also have decisions made by:

A. supported decision maker
B. caregiver
C. medical proxy
D. all the answers are correct
E. None of the answers are correct (CORRECT ANSWER)

Question/Answer(s) #3
In order to appropriately schedule patients with special healthcare needs for care, caregivers should communicate the following patient specific accommodations:
A. need for protective stabilization
B. request for therapy animal support
C. need for sensory reduction accommodation
D. all the answers are appropriate to communication (CORRECT ANSWER)

E. **Question/Answer(s) #4**
When establishing a transition process, it is important to gather the following information from the previous dental provider

A. previous history of in office sedation
B. behavioral accommodations during appointments
C. dental history
D. medical history
E. all of the answers are correct (CORRECT ANSWER)

Name of Primary Presenter Contact Susan Somerset DMD, Pharm D, MS
susan.somerset@cuanschutz.edu

Bio- Susan Somerset is an assistant professor at the CUSDM and co-director for the Special Care Dental Clinic. She is also a pharmacist. She completed her DMD at Temple University, a GPR at Christiana Care Medical Center and a Pharm D from USP. She has worked in hospital critical care units, drug information and global safety for pharmaceutical industry. Her experience in dentistry includes private practice, FQHCs and hospital dentistry. She lectures nationally on topics of pharmacology and special care dentistry.

Title: Active Learning: Encouraging Pathology Integrations Through Patient Box Questions

Authors: Tanya Wright, DDS, Jennipher Murphy, MS, and Catherine Flaitz, DDS, MS

Category: Faculty at the University of Colorado School of Dental Medicine

Successful learning includes promoting integrations/connections through relevant, but guided instruction, collaborative teamwork, and freedom of choice that reinforce important/interesting concepts. One active-learning approach is incorporating patient box question (PBQ) activities including clinically applicable material that reinforces new and previously learned material. The aim was to evaluate the perceived value of team-based, PBQ activities enhancing student learning of integrated general pathology content.

Methods: IRB review status is exempt. Eighty first-year dental students were randomly placed into 10 groups of eight and assigned topics discussed for each exam block. They were given a standardized patient scenario and asked to develop a question. The main criteria were: development of multiple-choice question; incorporation of new and previously assessed material; and justification of the correct answer using textbook references. Using a rubric, questions were graded by two course instructors with group feedback provided. At the end of the course, students were asked to give feedback on the PBQ activities. There were three self-reflection questions on the perceived value of the activities and relevance to their learning. Two questions applied the Likert 5-point scale ranging from strongly-agree to strongly-disagree and one free text for general comments. Descriptive statistics were used for analysis.

Results: Fifty-seven of the eighty students (72%) strongly-agreed/agreed incorporating previous topics for the PBQ assignment reinforced general pathology concepts compared to six (8%) disagreeing, while 68 students (90%) strongly-agreed/agreed for developing questions and writing justifications compared to two (3%) disagreeing. Positive feedback from open text comments included the collaborative spirit of working with others and making connections between various organ systems. Negative aspects included large group size and strict grading policy as defined by the rubric and guidelines.

Conclusion: The well-received, active-learning PBQ activities supported discovering connections and empowered students through collaboration, integration, and reinforcement of learned concepts in a complex didactic course.