39TH ANNUAL
RESEARCH DAY

Friday, February 23, 2024

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

DENTAL. INTEGRATED FOR HEALTH.

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

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39th Annual Research Day

Friday, February 23, 2024
Health Sciences Building, Elliman Conference Hall

8:30-10:30 am	**Poster set-up**
10:30-11:30 am	**Poster viewing and judging**
11:30-1:00 pm	**Lunch and Poster Social**
1:00-1:10 pm	**Welcome and Introduction:** Jeffrey Stansbury, PhD Senior Associate Dean for Research and Professor, School of Dental Medicine
1:10-1:15 pm	**Dean's Welcome:** Denise Kassebaum, DDS, MS, Dean, School of Dental Medicine
1:15-2:15 pm	**Introduction of Keynote Speaker: Timothy Cox, PhD, Professor** University of Missouri-Kansas City, School of Dentistry Title: *Insights into oral and craniofacial disorders in animal models through qualitative and quantitative high-resolution 3D imaging*
2:15-2:30 pm	**Trisani Affandi, PhD, Research Associate,** Dept of Craniofacial Biology, School of Dental Medicine Title: *Advances in Radioprotection of the Salivary Gland*
2:30-2:45 pm	**Abigail Mumme-Monheit, PhD candidate,** Dept of Craniofacial Biology, School of Dental Medicine Title: *Buffering Craniofacial Development*
2:45-3:15 pm	**Break and Refreshments**
3:15-3:45 pm	**Tamana Tiwari, BDS, MDS, MPH, Associate Professor** Department of Community Dentistry and Population Health, School of Dental Medicine Associate Director, Center for Oral Disease Prevention & Pop Health Research, School of Dental Medicine **Tanya Russell, PhD, Curriculum Manager** Center for Advancing Professional Excellent (CAPE), School of Medicine Title: *Patient-Centered Teaching: How have we done in the past seven years*
3:45-4:00 pm	**Kristin Watt, PhD, Assistant Professor** Department of Craniofacial Biology, School of Dental Medicine Title: *RNA Polymerase III in craniofacial cartilage and bone development*
4:00-4:15 pm	**Award announcements, continuing education QR-code, closing remarks**

39TH ANNUAL
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SPEAKER ABSTRACTS

Friday, February 23, 2024

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39th Annual SDM Research Day 2024

Friday, February 23, 2024

Keynote Speaker

Professor Timothy C. Cox

Insights into oral and craniofacial disorders in animal models through qualitative and quantitative high resolution 3D imaging

University of Missouri-Kansas City, School of Dentistry, Department of Oral and Craniofacial Sciences and Department of Pediatrics, School of Medicine

Dr. Timothy C. Cox is an Endowed Professor in Dental and Musculoskeletal Tissue Research at the University of Missouri Kansas City, School of Dentistry. He also holds a joint appointment in the School of Medicine's Department of Pediatrics. Dr. Cox earned his BS (with Honors) and PhD from the University of Adelaide, Australia, and undertook postdoctoral training in human genomics at both Baylor College of Medicine, Houston and the Telethon Institute of Genetics in Medicine (TIGEM) in Milan (Italy). He then returned to Australia (University of Queensland) to pursue his interests in Developmental Biology, before being given an opportunity to head his own group at the University of Adelaide. During this period, he was appointed as Director of Genetic Programs at The Australian Craniofacial Unit. In 2004, he moved to Monash University (Melbourne) before being recruited in 2006 to the University of Washington in Seattle, where he quickly rose to full professor in Pediatrics and Endowed Chair in Pediatric Craniofacial Research. He was lured to the UMKC Dental School in 2018. His research includes human genomics and both animal and cell-based model systems for understanding the role of gene variants in the presentation of common craniofacial conditions, such as cleft lip and craniofacial microsomia. Dr. Cox's lab has also become recognized as a leader in qualitative and quantitative high-resolution 3D tomographic imaging to assess the impact of genetic mutations and maternal diet on early facial morphogenesis and its postnatal consequences. He has published ~150 papers including prominent papers in Nature, Science Advances, Nature Genetics, Nature Communications, Developmental Cell and the New England Journal of Medicine. Dr. Cox has held numerous positions in international & national societies, including President of the Australia & New Zealand Society for Cell & Developmental Biology and Treasurer of the Society for Craniofacial Genetics & Developmental Biology (US). He has served on NIH Study Sections for over 14 years as well as reviewed for funding agencies in Europe and Australasia for over 25 years. Dr. Cox has published over 150 peer-reviewed research papers and his research laboratory has received continuous federal funding since 1997.

Dr. Cox will discuss how continued research into the understanding of genetic and epigenetic contributions to craniofacial development is paramount to obtaining new knowledge of common craniofacial conditions, such as cleft lip, midface hypoplasia and craniofacial microsomia. The utilization of animal model systems and quantitative high-resolution 3D tomographic imaging provides both qualitative and quantitative assessments of the impact of genetic mutations and maternal diet on early facial morphogenesis and its postnatal consequences.

Trisiani Affandi, PhD, Research Associate

Advances in Radioprotection of the Salivary Gland

University of Colorado School of Dental Medicine, Department of Craniofacial Biology

Patients treated with irradiation (IR) for head and neck cancer (HNC) often sustain collateral damage to non-tumor tissues in the oral cavity. Protein kinase C delta (PKCδ) regulates IR-induced apoptosis in salivary acinar cells, and inhibition of PKCδ preserves salivary gland function in mouse models of head and neck irradiation without protecting the tumor. *We hypothesize that inhibition of PKCδ suppresses apoptosis by increasing double stranded break (DSB) repair*. Formation of micronuclei was quantified to investigate genomic instability. DNA damage was analyzed by γH2AX foci quantification and Comet assay. *In vivo* fluorescent reporter assay was used to directly quantify non-homologous end joining (NHEJ) and homologous recombination (HR). Chromatin remodeling was investigated using a micrococcal nuclease (MNase) assay and by analysis of histone modifications using mass spectrometry. RPE cells that stably overexpress PKCδ show increased chromosomal instability, indicating that PKCδ has a negative impact on genome integrity. We show that endogenous PKCδ regulates chromatin accessibility and suppresses DSB repair. Contrarily, DNA repair is increased in PKCδ-depleted cells as evidenced by more rapid resolution of IR-induced γH2AX foci and a more rapid decrease in DNA damage. Depletion of PKCδ increases DNA repair through both NHEJ and HR pathways. Depletion of PKCδ is associated with increased MNase sensitivity, suggesting a more open chromatin, while overexpression of PKCδ decreases MNase sensitivity. In PKCδ-depleted cells, H3K23ac is decreased and H3K36me2 is increased, both histone marks are associated with DNA repair. Our data suggests a novel mechanism for control of apoptosis by PKCδ mediated through regulation of chromatin accessibility and DNA repair. Understanding the mechanism by which PKCδ regulates DNA damage-induced apoptosis may allow us to identify new targets for radioprotection of oral tissues in HNC patients.

This work is funded by the National Institute of Dental and Craniofacial Research F32 DE029116, R01DE015648, and R01DE027517; and the T32 Training Grant in Cancer Biology CA190216-03.

Ms. Abigail Mumme-Monheit, PhD Candidate

Buffering Craniofacial Development

University of Colorado School of Dental Medicine, Department of Craniofacial Biology– Anschutz Medical Campus, Cells, Stem Cells, and Development PhD program

This presentation will explain how we use zebrafish to study craniofacial development. I will cover broad concepts of developmental biology and explain how we can use our zebrafish system to better understand them. *MEF2C* mutations cause craniofacial phenotypes in humans, and mutations in the zebrafish gene *mef2ca* replicate these craniofacial phenotypes.

I will introduce the concepts of developmental canalization, incomplete penetrance, and variation. I will then explain how we can use these *mef2ca* mutants to better understand these concepts. We have used these mutants to deeply examine the relationship between phenotype severity and variation. Based on our analyses we have generated a novel quadratic model for the relationship between severity and variation. We found that wild-type conditions, as well as extreme severity resulted in low variance, whereas moderate severity was associated with high variation. In other words, we found that there was less constraint on the system upon destabilization by a mutant allele, leading to an increase in variation; however, when the most severe condition was reached, variation reduced because all individuals were maximally affected. These findings could have broad implications in a large array of contexts for understanding severity and variation in disease.

This research is funded by the National Science Foundation DGE 1938058.

Tamanna Tiwari, BDS, MDS, MPH, Associate Professor

University of Colorado School of Dental Medicine, Department of Community Dentistry and Population Health

Dr. Tamanna Tiwari is a researcher interested in behavioral health, health inequities, the integration of medical and dental care, and health informatic. She has extensively published her work in these areas. Dr. Tamanna Tiwari also serves as the Associate Director of the Center for Oral Disease Prevention and Population Health Research (COPPR) at the School of Dental Medicine.

Tanya D. Russell, PhD, Curriculum Manager

University of Colorado Anschutz Medical Campus, School of Medicine, Center for Advancing Professional Excellent (CAPE),

Dr. Tanya Russell joined the Center for Advanced Professional Excellence (CAPE) in 2015 and currently serves at the Curriculum Manager. Her background as a former research scientist enriches her role, overseeing daily curriculum and research activities, leading the CAPE curriculum team, and integrating simulation into healthcare education. Dr. Russell is passionate about addressing health disparities and promoting diversity, equity, and inclusion (DEI) in healthcare education. In her prior role as Simulation Education Project Coordinator, she effectively collaborated with faculty to enhance curricula and align CAPE services with evolving healthcare education, emphasizing DEI initiatives. She remains dedicated to advancing educational outcomes and addressing healthcare equity challenges through simulation.

Presentation: Patient-centered teaching: How have we done in the past seven years

Educational Objectives:

- Changes in the attitude and approach of the learner to the solution of dental problems; corrections of outdated knowledge
- Provision of new knowledge in specific areas
- Introduction to and/or mastery of specific skills and techniques
- Alteration in the habits of the learner; accurate educational objectives succinctly describe the education that will result from attending the course

- Understand the value that CAPE brings to dental education.
- Discuss trends in communication training for dental students over the last seven years.
- Discuss interactions between dental students and diverse patients.

Kristin Watt, PhD, Assistant Professor

RNA Polymerase III in cartilage and bone development

University of Colorado School of Dental Medicine, Department of Craniofacial Biology

Ribosome biogenesis and protein translation are essential processes required in all cells, yet disruptions in this process lead to tissue-specific human phenotypes which frequently affect craniofacial development. Pathogenic variants in genes encoding subunits of RNA Polymerase (Pol) III lead to a variety of phenotypes including hypomyelination in the central nervous system, premature aging, hypodontia, and other rare anomalies of the head and skull. Pol III transcribes 5S ribosomal RNA (rRNA), which is a critical component of the ribosome, in addition to other noncoding RNAs including tRNAs. Given the perturbed development of neural crest cell (NCC) derived tissues in humans with pathogenic variants in Pol III, including teeth and craniofacial bones, we hypothesized that Pol III-mediated transcription is important for craniofacial development through the regulation of ribosome biogenesis and translation. We performed a tamoxifen-inducible conditional deletion of Pol III subunit *Polr3b* at postnatal day 2 in mice to understand the role for Pol III in the growth of craniofacial bones. Micro-CT analysis demonstrated a significant difference in the size of the mandible and revealed a reduction in the growth of the incisor. To explore the requirement for Pol III at earlier developmental stages, zebrafish with a mutation in Pol III subunit *polr3a* were established. These homozygous mutant zebrafish display hypoplasia of the craniofacial cartilage, bone, and pharyngeal teeth, and are lethal at larval stages. Together, these zebrafish and mouse models indicate Pol III functions in the growth and development of craniofacial cartilage, bone and teeth at larval and postnatal stages. Current and future work aims to understand Pol III-mediated transcription during cranial NCC development and differentiation and the mechanisms that drive the growth and development of craniofacial cartilage, bone, and teeth.

This work was in part funded by the National Institute of Dental and Craniofacial Research K99DE030971.

Easel #	Name/First Author	Title
1-DDS/ISP	Nisali Piyasena	Assessing *Streptococcus mutans* Susceptibility to Azobenzene Polymers
2-DDS/ISP	Kaylee Appley	A Novel Urethane Monomer to Achieve High-Performance Photopolymers
3-DDS/ISP	Matt Guralsky	Investigation of Acidic Comonomers to Achieve High Performance with Urethane Monomers
4-DDS/ISP	Elle M. Bertuccelli	Investigating the role of *gata3+* neural crest cells in craniofacial development
5-DDS/ISP	Summer Booth	Theobromine slows enamel erosion and lesion progression
6-DDS/ISP	Joshua Crane	Fluoride Enhanced Orthodontic Elastics: Evaluating Prevention of Demineralization and Erosion
7-DDS/ISP	Madison Watt	The Human Papillomavirus, Head & Neck Cancer, and the Latest Vaccine Considerations for Oral Health Professionals
8-DDS/ISP	Hunter Pine	Tooth Loss in an Aging Population as it Relates to Systemic Health
9-DDS/ISP	Monika Reddy	Evaluation Report: School-Based Health Center- Oral Health Programs
10-DDS/ISP	Ansha Bharath	A Novel Resin Based Endodontic Filling Material
11-DDS/ISP	Karminder Singh	A comparison of the efficacy of different root canal irrigants in removing intracanal calcium hydroxide dressing—a scanning electron microscopic cleanliness evaluation
12-DDS/ISP	Sadaf Fadakar	The *PocketPerio* application significantly increases the accuracy of diagnosing peridontal conditions
13-DDS/ISP	Devan Cruz	A novel method for grading pre-clinical restorations using a digital workflow
14-GS	Thomas Forman	PDGFRα signaling regulates Srsf3 transcript binding to affect PI3K signaling and endosomal trafficking
15-GS	Cassandra Minne	Alternative RNA splicing of transcripts encoding protein serine/threonine kinases downstream of PDGFR signaling in the facial mesenchyme
16-Post	Jennyfer Mitchell	Alx Function in the Frontonasal Skeleton Revives the Pharyngeal Arch-0 Hypothesis
17-Post	Humberto Escobedo	Polymer Coatings to Combat Microbial Growth on 3D Printed Denture-Base Materials
18-Post	Stanley Kanai	Characterization of calcium signaling in cranial neural crest cells during lower jaw development
19-Post	Maria Campana	PDGFRα/β heterodimer activation negatively affects downstream ERK1/2 signaling and cellular proliferation
20-Post	Raisa Bailon	Fins come and fins go: The *smoothback* zebrafish mutant informs median appendage development.
21-Post	Paul Rolfes	Differences in funding approval for standardized Medicaid orthodontic treatment cases submitted to different Medicaid jurisdictions: a controlled prospective survey
22-Staff	Austyn Salazar	Tuning Inkjet Photopolymer Formulations for Enhanced Properties
23-Staff	Colette Dolby	A Role for *prrx1* Genes in Median Fin Development
1 FAC	Yanira Owens	Examining Burnout between Traditional and International Dental Students Training in the U.S.

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STUDENT ABSTRACTS (DDS AND ISP)

Friday, February 23, 2024

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Title: Assessing *Streptococcus mutans* Susceptibility to Azobenzene Polymers

Authors: Nisali Piyasena[1], David Danforth[2] Devatha Nair[1,3] and Michael Schurr[2],

[1]School of Dental Medicine, University of Colorado Anschutz Medical Campus

[2]School of Medicine, Department of Immunology and Microbiology, University of Colorado Anschutz Medical Campus

[3]Department of Craniofacial Biology, University of Colorado Anschutz Medical Campus

Category: Dental Students

Purpose:

This study aims to understand the inhibitory effects of acrylated hydroxyazobenzene copolymers (AHA copolymers) on *S. mutans* , specifically with iron. This will further progression towards utilizing the copolymers in dental materials. Colony-Forming Unit (CFU) assays and Geometric Viability Assay (GVA) are utilized to quantify bacterial growth.

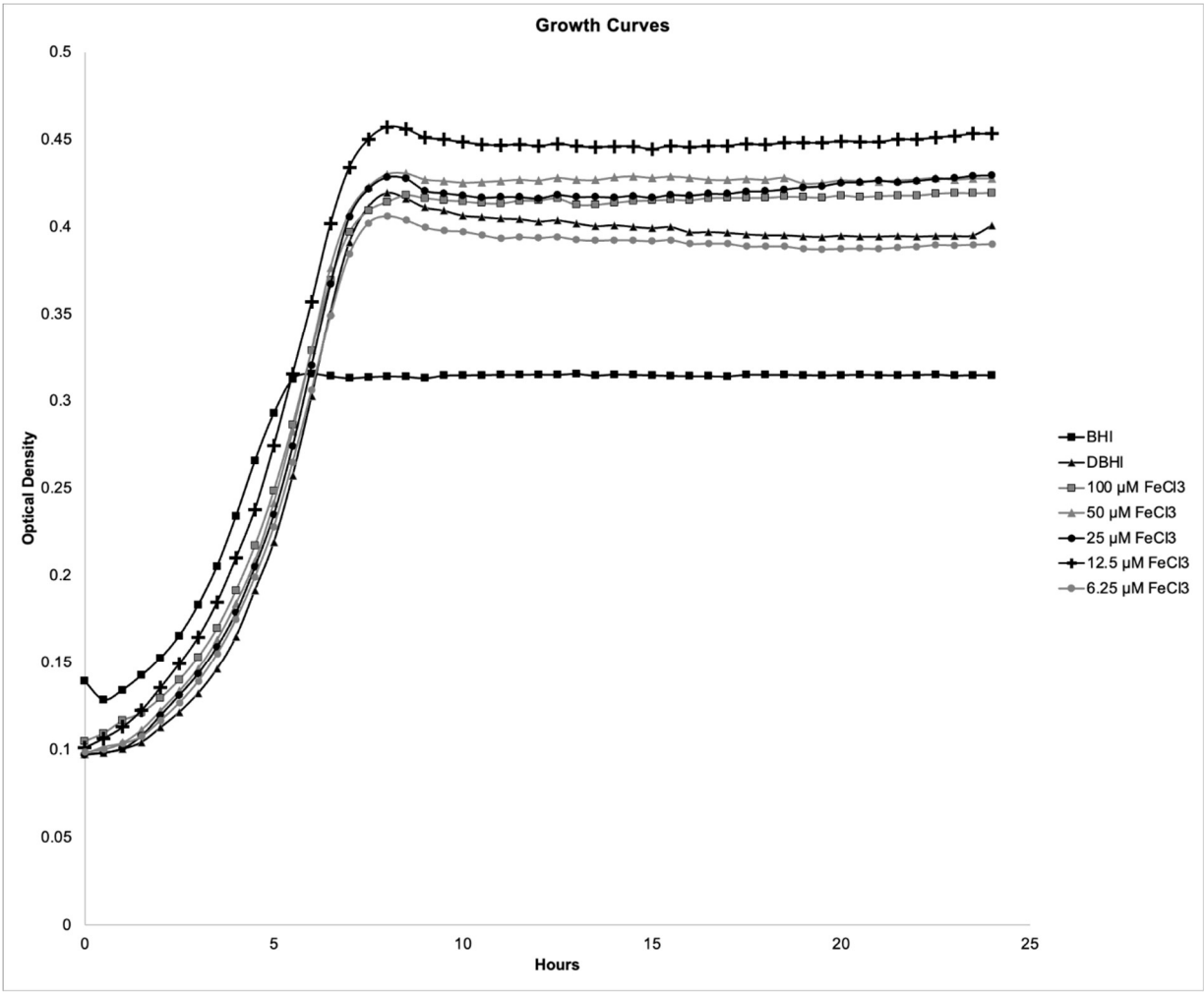
Methods:

S. mutans was grown in brain-heart infusion (BHI) broth or dialyzed BHI (dBHI), in which Fe^{3+} is removed. The optimal iron concentration for bacterial growth was determined using optical density measurements at 600 nm of dBHI with varying $FeCl_3$ concentrations for 24 hours in a Synergy H1 microplate.

Samples composed of 70:30 molar weight of BisGMA:TEGDMA with and without 2wt% AHA copolymer of dimensions 0.8mm x 4.8mm were added to samples of dBHI with varying $FeCl_3$ concentrations. These were incubated for 8 hours in a 37°C incubator containing 5% CO_2. Hourly,10 µL were removed from each sample, serially diluted using PBS, and plated on BHI plates. The CFUs were quantified after 40 hours of incubation.

This work was funded by the National Institute of Dental and Craniofacial Research K25DE027418.

Results:



The results show that *S. mutans* in dBHI with 12.5 µM $FeCl_3$ grew to the highest density.

Conclusion:

Fe^{3+} content impacts *S. mutans* growth. The results indicate BHI contains iron concentrations that inhibit growth. The organism grew better in dBHI with varying iron concentrations.

The CFU counts of dBHI + AHA copolymer were 5.60E+04 ± 1.14E+04 with $FeCl_3$, while control samples without $FeCl_3$ were 7.30E+04 ± 4.58E+03, indicating the contributory role of Fe^{3+} content on AHA copolymer's inhibitory response.

Title: A Novel Urethane Monomer to Achieve High-Performance Photopolymers

Authors: Kaylee Appley, Austyn Salazar, Jeff Stansbury

Category: Dental student (DS1)

Purpose: The purpose of this study was to test and compare a newly synthesized urethane monomer to a previously characterized experimental material.

Methods: Samples of 2x2x25 mm were photocured using a 365nm light at an intensity of 100 mW/cm^2. Conversion was tracked via near-FTIR using the 6150 cm^{-1} peak corresponding to acrylates. Formulations were made by weighing urethane monomers out and then using a molar ratio of 1:1 acid:urethane along with 0.1 wt% 2,2-dimethoxy-2-phenylacetophenone (DMPA) as a photoinitiator. Samples were cured for 120 seconds twice and then put into a CureBox to post-cure further at 80°C for 1 hour under 365/405 nm irradiance.

Results: Urethane dimethacrylate (UDMA) and methacrylic acid (MAA) were used as a comparative model formulation and trihydroxy diurethane dimethacrylate as a novel urethane monomer was combined with either MAA or acrylic acid (AA). It can be seen that the flexural strength between UDMA and THDUDMA both with MAA are reasonably comparable, however the UDMA + MAA has a higher average at 184.7 MPa compared to 149.4 MPa for the THDUDMA + MAA. Contrary to the flexural strength, the modulus of THDUDMA + MAA far exceeds that of UDMA + MAA reaching 6.66 GPa. Additionally, using AA with THDUDMA pushes both flexural strength and modulus well above that of UDMA + MAA reaching a strength of 231.8 MPa and modulus 6.74 GPa, respectively.

Urethane Monomer	Acidic Comonomer	Flexural Strength (MPa)	Modulus (GPa)	Toughness (MPa)
UDMA	MAA	184.7 (23.15)	4.12 (0.33)	10.33 (6.4)
THDUDMA	MAA	149.4 (52.27)	6.66 (0.66)	2.02 (1.57)
	AA	231.8 (35.61)	6.74 (0.25)	6.21 (3.12)

Conclusion: THDUDMA was synthesized, tested, and compared to properties of UDMA to see how it performed. The modulus of THDUDMA with MAA or AA outperformed that of the UDMA/MAA system, however, only the THDUDMA/AA formulation achieved higher flexural strengths. UDMA produced higher toughness. The study thus far showed that THDUDMA could be a high-performance urethane monomer, but more studies are needed to further enhance toughness as well as strength and stiffness.

This work was funded by the National Institute of Dental and Craniofacial Research R21DE032797.

Title: Investigation of Acidic Comonomers to Achieve High Performance with Urethane Monomers

Authors: **Matt Guralsky**, Austyn Salazar, Jeff Stansbury

Category: Dental student (DS1)

Purpose: The purpose of this study is to find suitable acidic monomers that achieve similar mechanical reinforcement properties when used with urethane comonomers that have been previously observed using (meth)acrylic acid ((M)AA).

Methods: Samples were photocured with conversion tracked via near-FTIR. Formulations were prepared with comonomers using a molar ratio of 1:1 or 2:1 acid:urethane along with a photoinitiator. Samples were cured for 120 seconds twice and then put into a CureBox to post-cure further at 80°C for 1 hour with 365/405 nm irradiance.

Results: Diurethane dimethacrylate (UDMA) was used as the initial urethane monomer due to its relevance in the dental field. It was tested as a homopolymer and achieved decent mechanical properties. However, previous studies have shown that the inclusion of MAA lowered viscosity and enhanced polymer mechanical properties. The jump in modulus from 3.12 to 4.12 GPa as well as the increase in flexural strength from 158.5 to 184.7 MPa shows the advantages of an acidic comonomer. Other acidic comonomers were tested with UDMA such as mono-2-(methacryloyloxy)ethyl maleate (MaleateMA) and mono-2-(methacryloyloxy)ethyl succinate (SuccinateMA). The flexural strength of the formulations using these acidic comonomers both have comparable flexural strengths to that of the UDMA homopolymer but each attain a higher modulus at 3.69 (MaleateMA) and 3.57 GPa (SuccinateMA). More acidic comonomers are to be tested such as a mono-2-(methacryloyloxy)ethyl itaconate (ItaconateMA) as well as using these with a monourethane dimethacrylate (MUDMA). MUDMA + MAA has shown favorable properties and the addition of these acidic comonomers would be beneficial to test further.

Urethane Monomer	Acidic Comonomer	Flexural Strength (MPa)	Modulus (GPa)	Toughness (MPa)
UDMA	-	158.45 (5.79)	3.12 (0.12)	9.25 (1.64)
	MAA	184.73 (23.15)	4.12 (0.33)	10.33 (6.4)
	MaleateMA	155.86 (7.94)	3.69 (0.34)	-
	SuccinateMA	146.67 (9.64)	3.57 (0.2)	-
MUDMA	MAA	181.3 (16.5)	3.7 (0.16)	8.72 (2.77)
DUDMA	MAA (2x)	224.24 (24.62)	6.14 (0.28)	5.21 (1.35)
	AA (2x)	228.87 (22.96)	5.67 (0.69)	13.27 (6.76)

Conclusion: During this investigation, multiple acidic comonomers were combined with UDMA with the homopolymer and copolymers compared. Mechanical properties

achieved from these formulations did not perform as well as MAA but did achieve higher a modulus than UDMA as a homopolymer. Further investigations with alternative urethane monomers and acidic comonomers will be conducted to better understand useful combinations.

This work was funded by the National Institute for Dental and Craniofacial Research - R21DE032797.

Title: Investigating the role of *gata3*+ neural crest cells in craniofacial development

Elle M. Bertuccelli, Stanley M. Kanai, MaCalia R. Augustus, James T. Nichols and David E. Clouthier

Category: DDS/ISP

Mutations in the gene *GATA3* are associated with craniofacial birth differences such as hemifacial microsomia and choanal atresia, in addition to hypoparathyroidism, sensorineural deafness, renal dysplasia (HDR) syndrome in humans. However, it is unclear how loss of *GATA3* causes these disorders. Single cell RNA-sequencing has revealed a previously uncharacterized cluster of cranial neural crest cells (NCCs) that express *gata3, gata2a*, *sost*, and *isl2a*. To understand the significance of these cells, we began by identifying the location of *gata3*+ expressing cells in the developing zebrafish embryo. Our long-term goal is to understand how these *gata3*+ cells contribute to zebrafish craniofacial development and how this relates to *GATA3*-associated craniofacial birth differences in humans.

Methods: We performed fluorescence in situ hybridization (ISH) on whole-mount zebrafish embryos to visualize the expression patterns of *gata3, gata2a, sost, and isl2a*. To generate ISH probes, we cloned fragments of cDNAs for the four genes of interest and then performed in vitro transcription to synthesize digoxigenin-labeled RNA probes. We then performed fluorescence ISH on *fli1a:GFP* transgenic zebrafish embryos at 28 hours post fertilization. The embryos were imaged with confocal microscopy.

Results: We identified a population of *fli1a:GFP*-labeled NCCs in the frontonasal region of the head that expresses all four genes of interest. *gata3* expression was broad, spanning the medial frontonasal region to a region ventral to the optic fissure. *gata2a* expression was confined to the lateral frontonasal region. *isl2a* expression was confined to the medial frontonasal region and *sost* expression was further restricted medially.

Conclusion: Our data show that the novel *gata3*+ NCC cluster corresponds to a frontonasal population of NCCs. Based on past lineage tracing studies, this population likely contributes to the trabecular cartilage of the cranial base. Future work will test this hypothesis and determine the roles of *gata2a, isl2a,* and *sost* in this process.

Abstract Title: Theobromine slows enamel erosion and lesion progression

Authors: Summer Booth and Clifton Carey

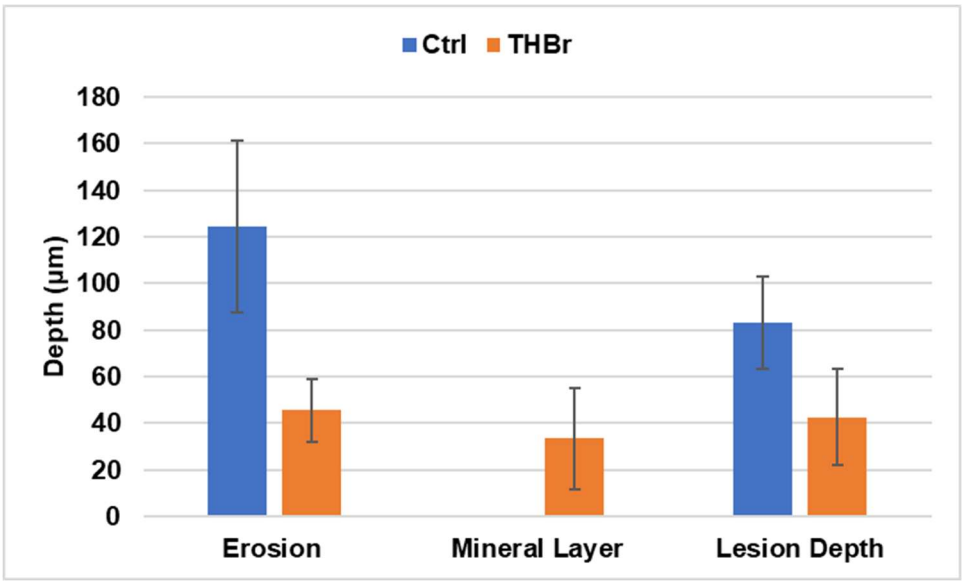
Category: DDS/ISP

Objective: The objective of this study was to determine if 300ppm theobromine has the ability to prevent enamel erosion and/or to prevent lesion progression. We hypothesized that a 300ppm theobromine rinse would prevent enamel demineralization and erosion compared to a non-theobromine rinse.

Methods: Human enamel samples were cut into (2x2x1)mm blocks and mounted on plastic blocks. The natural surface of each sample was bisected into a lacquer protected and a non-treated area. The samples (n=10/group) were placed in a modified Featherstone model mouth system that simulated a realistic salivary flow with 3x/day lactic acid challenges. During the 5-day trial, the test group (THBr) received a 2x/day 300mmol theobromine rinse and the control group (Ctrl) received a water rinse 2x/day. The enamel samples were then cross sectioned, microscopically imaged and analyzed for erosive loss and lesion depth using Image-J software.

Results: In this mouth model 2x/day exposure to a rinse of 300ppm theobromine significantly reduced the amount of enamel erosion {THBr=46±14μm vs Ctrl=124±37μm} at $p<0.0001$; and also significantly reduced the lesion depth {THBr=43±20μm vs Ctrl=83±20μm} at $p=0.0003$. For the THBr samples only, a mineralized layer 33±22μm thick was observed at the surface of the lesion.

Conclusions: The data supports our hypothesis that the theobromine rinse has a significant protective effect on the demineralization of enamel. Further studies are needed to confirm these results and to analyze the composition of the mineralized layer observed on the surface of the lesion.

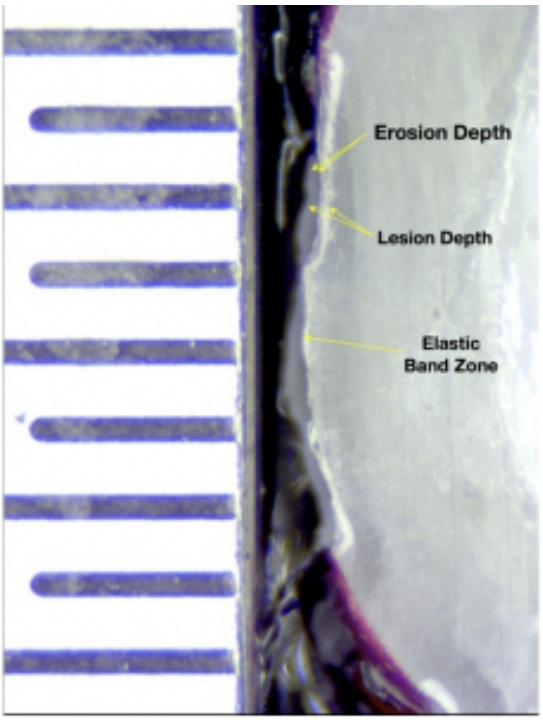

Ctrl
THBr
180
160
140
120
100
80
60
40
20
0
Depth (µm)
Erosion
Mineral Layer
Lesion Depth

Abstract: 2024 CU Research Day - Josh Crane

Title: Fluoride Enhanced Orthodontic Elastics: Evaluating Prevention of Demineralization and Erosion

Authors: J. Crane, R. Davis, C. Carey

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



Purpose: This in-vitro study assessed the efficacy of fluoride enhanced inter-arch latex elastic bands for the prevention of human enamel demineralization and erosion. We hypothesized that the concentrations of fluoride released by fluoridated elastics into salivary flow will mitigate demineralization and erosion of enamel during orthodontic treatment. Both sodium fluoride (NaF) and sodium monofluorophosphate (NaMFP) coated elastics, as well as a control elastic, were evaluated (n=8 per group).

Methods: Orthodontic latex elastics were coated with 5% w/w NaF or NaMFP mixed into latex monomer (Copydex) and cured in 3% acetic acid. The controls for the experiment were untreated elastics. The elastics were placed around enamel blocks (2x2mm) cut from healthy human teeth. These were placed in the modified Featherstone model mouth system that simulates continuous salivary flow and periodic demineralization lactic acid challenges. Elastics were changed twice daily (morning and evening), the duration of each trial was 14 days. Enamel samples were then cross-sectioned and microscopically imaged for evaluation of demineralization and erosion using Image-J software.

Results: NaF and NaMFP elastic bands significantly reduced both lesion depth and erosion depth relative to controls (ANOVA $p<0.05$). NaF reduced both lesion depth and erosion depth more effectively than NaMFP (t-test $p<0.05$). There was a notable protective effect of the bands directly beneath the elastics for all experimental groups.

Conclusions: The data supports our hypothesis that there is a significant preventive effect of the fluoridated elastic bands on human enamel samples.

The Human Papillomavirus, Head & Neck Cancer, and the Latest Vaccine: Considerations for Oral Health Professionals

Madison Watt[1], Emily Li DDS[2], James Closmann DDS[3], Oliver Chang MD[4]

Category 1: Dental students and ISP students at the School of Dental Medicine

Purpose

Human papilloma virus (HPV) is associated with both benign and malignant disorders including genital warts and a variety of cancers including oropharyngeal squamous cell carcinomas (OPSCCs). This project examines the literature regarding developments in the HPV vaccination schedule and its relevance to the prevention of head and neck cancer.

Methodology

We searched various medical databases using keywords such as “HPV OPSCC”, “HPV oropharyngeal cancer”, and “HPV oral cancer” to collect the most recent information on this topic.
Histology slides were prepared, analyzed, and captioned by Dr. Oliver Chang MD at the University of Washington School of Medicine.

Results

HPV has been associated with a variety of head and neck cancers including those of oropharynx, which have increased considerably in the past 20 years. Currently, the 9-valent Gardasil 9 is the only vaccine being distributed and is recommended for both men and women for the prevention of HPV and associated malignancies.

HPV-associated OPSCCs exhibit pathophysiologic and histologic differences from HPV-negative OPSCCs and have generally more positive outcomes, so de-escalation approaches are being considered in the treatment of these cancers to preserve health of the patient and minimize long-term toxicities.
The dentist may help play a role in the prevention of HPV-related OPSCCs by communicating with the patient about vaccination recommendations and preforming in-office cancer screenings, but further education is needed to ensure adequate health literacy for the most effective communication.

Conclusions

1. Some strains of HPV are strongly associated with oropharyngeal squamous cell carcinoma.
2. These cancers are preventable with the Gardasil 9 vaccine.
3. HPV-associated OPSCCs have different clinical presentations and responses to treatment than those not associated with HPV.
4. It is a responsibility of the dentist to screen for these cancers, as well as educate the patient on prevention strategies for HPV.

Title: Tooth Loss in an Aging Population as it Relates to Systemic Health

Authors: Hunter Pine, Monika Reddy, Rachel Johnson, Nhi Nguyen and Tamanna Tiwari

Category: DDS/ISP

Purpose: This study examined the associations between CVD, diabetes, tobacco use, and functional dentition in adults (≥55 years of age) over six years and evaluated a six-year trend in the factors associated with tooth loss.

Methods: Cross-sectional data was collected from patients seen at the university clinic of Colorado School of Dental Medicine clinics between 2017 and 2022. Electronic health records were reviewed for age, sex, race/ethnicity, self-reported medical histories of cardiovascular disease (CVD) and diabetes, and social history of tobacco use. The outcome variable was the number of natural teeth present in the oral cavity (<20 teeth: yes/no). Univariate and multivariable logistic regressions were performed to test the association between the outcome variable and the independent variables CVD, diabetes, and tobacco use. Analysis was performed on data from each year, as well as the data as a whole.

Results: Of 4338 patients, 36.2% had <20 teeth. There were greater odds of tooth loss in patients reporting CVD (OR=1.18, 95% CI=1.02, 1.37, p=0.027), diabetes (OR=1.25, 95% CI= 1.05, 1.48, p=0.012), and tobacco use (OR=1.74, 95% CI=1. 43, 2.11, p<0.0001). An overall stable trend in tooth loss odds was seen in patients reporting diabetes and tobacco use over six years.

Conclusion: This study suggests evidence of sustainable systemic-oral connections over six years in a university-based older adult patient population. Lack of functional dentition was significantly associated with tobacco use and diabetes, elevating the importance of training in systemic-oral connections for dentists and medical-dental integration.

Name: Monika Reddy Bhuma
Title: Evaluation Report: School-Based Health Center- Oral Health Programs
Category: DDS/ISP

The Project SBHC-SBOH strategy aims to evaluate oral health services within school-based health centers (SBHCs). The strategy strives to ensure improved access and oral health outcomes for students. The report primarily focuses on presenting findings from the planning/discovery phase, offering insights into the populations the SBHC sites serve, potential obstacles, and success stories.

Nine interviews were undertaken with several stakeholders from different SBHC sites. In-depth interviews captured a diverse range of perspectives from the participants.

Stages of Program Implementation:
The report segmented SBHCs into three categories based on their readiness for the SBHC-SBOH program:

- **Beginner:** Priorities are hiring qualified staff and acquiring essential equipment and space.
- **Intermediate:** Resolving ongoing staffing needs, consolidating infrastructure, and maintaining cost efficiency.
- **Advanced:** Fully equipped and staffed, these centers focus on best practices and sustained high-quality care delivery.

Key Findings:

- **Populations Served by the SBHC-SBOH Program:** Sites majorly support communities that face oral health disparities, especially low-income, predominantly Hispanic or Spanish-speaking, have food insecurity, or high disease burden.
- **Barriers to Implementation of Oral Health Program:** Obstacles include funding mandates, school-related issues, staffing concerns, the limitations of electronic health records, and a fluctuating workforce.
- **Facilitators and Measurement of Success:** Collaboration with teachers, school boards, and students, integration of diverse health services, plans for infrastructure enhancement, and interprofessional cooperation were highlighted as major success drivers.
- **Success in the Implementation of the SBHC-SBOH Program:** Success markers varied across sites but were predominantly tied to accomplishing dental procedures, closing referral loops, and fostering interprofessional cooperation. Some stakeholders also emphasized broader objectives, like establishing comprehensive dental homes for children.

Conclusion:

This report illuminates the various challenges and opportunities encountered by SBHCs in their quest to improve oral health outcomes. The findings lay the groundwork for enhancing the SBHC-SBOH program, underscoring the importance of tailored strategies based on readiness levels. Through focused efforts addressing the outlined challenges, the SBHC-SBOH initiative has the potential to drastically improve the oral health of school-going children.

A Novel Resin Based Endodontic Filling Material

A. Bharath, A. Salazar and J.W. Stansbury
Category: ISP-1 Student at the School of Dental Medicine

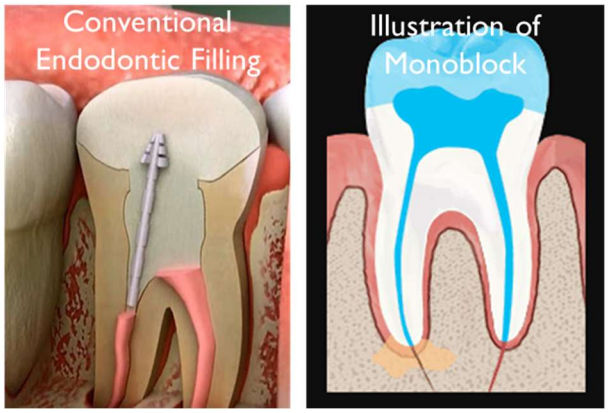
Purpose: A caries-infected tooth can lead to infection of the periapical tissue and, without timely intervention, can progress to significantly more serious complications. Following debridement and disinfection, conventional Endodontic procedures have used a gutta percha filling material and a separate sealer to restore the tooth. However, even following final crown placement this approach can lead to vertical tooth fracture due to insufficient strength of the filling material and possible dentin dehydration. To address this, the present study considers an Endodontic Monoblock approach to connect the dentin 'wall-to-wall'. A novel resin-based material was developed with the following aims: 1) Chemically bond to both radicular and coronal dentin, 2) Reduce internal stress concentration within the tooth by minimizing different material interactions and, 3) Achieve mechanical properties similar to Dentin.

Methods: Resin (ThTUDMA) and Glass Ionomer components were mixed with varying percentages of monomeric and polymeric acrylic acid, a crosslinking agent and 40 vol% of water. Combinations achieving greater than 90 % resin-phase conversion were subjected to 3-point bend testing following storage dry, in water or in artificial saliva. Additionally, micro-tensile tests were conducted to assess bonding to Dentin.

Results: From over 30 formulations, the best performing showed an average flexural strength of 174.5 MPa and Young's Modulus of 15.71 GPa on dry storage. The average Dentin bond strength was 11.6 MPa. When stored wet, the average strength and stiffness reduced to 19.2 MPa and 1.58 GPa, respectively.

Conclusions: Overall, the outstanding properties under dry storage conditions can be attributed to the hydrophilic compound forming an unusually strong resin matrix while the ion exchange with the Glass Ionomer enables bonding to the Dentin. The reduction when the samples were stored wet may be due to excessive water uptake that could be mitigated when the material is confined by a tooth, which requires further study.

a)



b)

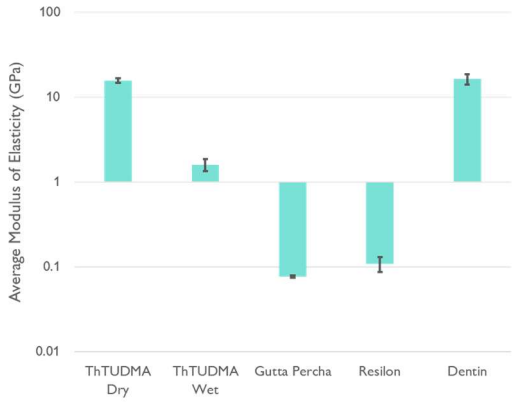


Figure1: a) Comparison between conventional, multi-material and Monoblock Endodontic treatment; b) Illustrative results demonstrating the measured average and ranges of modulus of elasticity for the novel resin-based material compared to existing materials and Dentin

This work was funded by the National Institute for Dental and Craniofacial Research -R21DE032797.

ABSTRACT FOR POSTER

Author: Karminder Singh

Category- DDS/ISP1

Aim: A comparison of the efficacy of different root canal irrigants in removing intracanal calcium hydroxide dressing—a scanning electron microscopic cleanliness evaluation.

Methods: Fifty-four single-rooted mandibular premolars were selected, canals were instrumented till Protaper Gold F3, and the No. 30 K file was used as the master apical file (MAF) and dressed with calcium hydroxide. $Ca(OH)_2$ medication was removed by 5 different experimental groups: 5.25% NaOCl (G1), EDTA-C (G2), 15% citric acid (G3), EDTA-T (G4), and re-instrumentation with MAF using NaOCl and lubrificant, followed by EDTA-T (G5). The roots were analyzed under a scanning electron microscope in the cervical, middle, and apical thirds (9, 6, and 3mm from the apex). Statistical analysis was performed using the Mann-Whitney U test and Kruskal-Wallis ANOVA at the 5% level of significance.

Results: Group G5 had the best results in all thirds, with significant statistical differences compared to all other groups in the middle and coronal third and to G1 in the apical third. On the other hand, G1, only flushed with NaOCl, had the worst results, with statistical differences in all thirds compared to the other groups. The recapitulation of MAF in combination with irrigants improved the removal of calcium hydroxide medication better than an irrigant flush alone.

Conclusions: None of the irrigating solutions used in this study were efficient in completely removing the calcium hydroxide from the root canals. EDTA-T,a combination of a chelating agent and 0.2% sodium lauryl ether sulfate (Tergentol) biological detergent,showed the best result,which apparently improved its efficacy in eliminating calcium ions and wall debris. However, it is also essential to clean the canal wall using the last apical file with sodium hypochlorite and lubricant before the final flush to improve the removal of calcium hydroxide medication.

Title: The *PocketPerio* application significantly increases the accuracy of diagnosing periodontal conditions.

Authors: Sadaf Fadakar,[1] David K. Okano,[2] Sangeetha Chandrasekaran,[3] Yoolim Kim,[3] Tonia C. Carter,[4] Neel Shimpi,[5] Nikola Angelov[6], and Karo Parsegian.[3,6]

Category for competition: Dental and ISP students at the School of Dental Medicine.

Affiliations:
[1]Predoctoral dental student, School of Dental Medicine, University of Colorado Anschutz Medical Campus (Aurora, CO, USA)

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Abstract

Purpose: To develop the software to assist with diagnosing periodontal conditions and determine its diagnostic accuracy.
Methods: All experimental protocols were approved by the Committee for the Protection of Human Subjects of the Institutional Review Board (IRB) of the University of Texas Health Science Center at Houston (UTHealth, #HSC-DB-18-0663) and Colorado Multiple IRB of the University of Colorado Anschutz Medical Campus (CU, #22-2206). The web-based *PocketPerio* application was developed using the Flutter software development kit and the Dart programming language. At UTHealth, 22 third-year dental students (DS3) diagnosed ten cases with (test) and without *PocketPerio* (control) during a mock examination. At CU, 105 DS3, 13 fourth-year dental students (DS4), and 32 senior second-year International Student Program (ISP2) dental students used *PocketPerio* to assist in diagnosing periodontal conditions chairside. The participants also provided optional anonymous feedback on *PocketPerio* using Qualtrics^{XM} software. Statistical analysis was performed using a non-parametric paired two-tailed test of significance with the Wilcoxon matched-pairs signed rank test. The null hypothesis that *PocketPerio* did not affect periodontal diagnosis accuracy was rejected at a <0.05.
Results: Periodontal diagnoses made using *PocketPerio* correlated with those made by periodontics faculty ("gold standard") in all cases. During the mock examination, *PocketPerio* significantly increased the accuracy of diagnosing periodontal conditions compared to the control (52.73 vs. 13.18%, respectively). Chairside, *PocketPerio* significantly increased the accuracy of primary (100 vs. 40.0%) and secondary (100 vs. 14.25%) periodontal diagnoses compared to the respective controls. Test students, regardless of their year of training, felt more confident using *PocketPerio* to assist in diagnosing periodontal conditions than their current tools. Overall, the participants provided positive feedback on *PocketPerio* and provided constructive criticism.
Conclusions: Dental students demonstrated significantly higher accuracy and confidence in diagnosing periodontal conditions using *PocketPerio*. Dental providers can use *PocketPerio* as an adjunct tool in diagnosing periodontal conditions.

Title: A novel method for grading pre-clinical restorations using a digital workflow
Authors: Devan S. Cruz, Thomas Greany, Elle M. Bertuccelli, John Esquivez, Simon Monley, Ahmad Alfaresi

Category: DDS/ISP

Purpose: Traditional preclinical dental grading methodologies suffer from various forms of human errors. Objective, consistent assessment remains a goal. In this experiment, we aim to explore a novel way of grading pre-clinical operative dentistry restorations objectively, consistently, and according to a biologically determined reference standard using intraoral scanning and 3D modeling techniques.

Materials and methods:

Class II DO preparations and restorations were performed on Standard Kilgore 200 typodont teeth (#30) and (#12) (n=50). The typodonts were scanned using the Trios scanner, typodont teeth were prepared and restored with Filtek Supreme composite, and typodonts were re-scanned. CloudCompare software was used to measure discrepancies between student fillings and the ideal standard prior to preparation and restoration. Following this, 10 randomly selected restored teeth from our sample were given to 3 faculty members, who were asked to grade each tooth according to the rubric used in operative dentistry courses. Accuracy and consistency of grading each tooth between faculty members was assessed and compared to the results from the CloudCompare software.

Results:
A novel method for inspecting student-prepared and restored typodont teeth was successfully developed, using extant intraoral scanning technology to acquire high resolution surface contour data. Teeth were compared before and after using open source analysis software. A grading rubric was developed which will be refined by comparison with standard visual inspection methods. A visual analog scale was developed to provide immediate student feedback.

Conclusion:

Our results indicate that intraoral scanning and digital analysis improves accuracy and removes biases from grading. Improved grade validity promises to improve student morale and instructor trust. Additionally, the process of using digital grading methods decreases the time spent on grade and feedback dissemination. Future research is indicated to explore whether digital assessment can produce the same results when grading crown preparations, provisional crown fabrication, and esthetic dentistry.

39TH ANNUAL
RESEARCH DAY

GRADUATE STUDENT ABSTRACTS

Friday, February 23, 2024

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

DENTAL. INTEGRATED FOR HEALTH.

PDGFRα signaling regulates Srsf3 transcript binding to affect PI3K signaling and endosomal trafficking

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Category: Graduate Students

Purpose: Signaling through the platelet-derived growth factor receptor alpha (PDGFR α) plays a critical role in craniofacial development, as mutations in *PDGFRA* are associated with cleft lip/palate in humans and *Pdgfra* mutant mouse models display varying degrees of facial clefting. Phosphatidylinositol 3-kinase (PI3K)/Akt is the primary effector of PDGFRα signaling during skeletal development in the mouse. We previously demonstrated that Akt phosphorylates the RNA-binding protein serine/ arginine-rich splicing factor 3 (Srsf3) downstream of PI3K-mediated PDGFRα signaling in mouse embryonic palatal mesenchyme (MEPM) cells, leading to its nuclear translocation. We further showed that ablation of *Srsf3* in the murine neural crest lineage results in severe midline facial clefting, due to defects in proliferation and survival of cranial neural crest cells, and widespread alternative RNA splicing (AS) changes. Here, we sought to determine the molecular mechanisms by which Srsf3 activity is regulated downstream of PDGFRα signaling to control AS of transcripts necessary for craniofacial development.
Methods and Results: We demonstrated via enhanced crosslinking and immunoprecipitation (eCLIP)-seq of MEPM cells that PDGF-AA stimulation leads to preferential binding of Srsf3 to exons and loss of binding to canonical Srsf3 CA-rich motifs. Through the analysis of complementary RNA-seq data, we showed that Srsf3 activity results in the preferential inclusion of exons with increased GC content and shorter flanking introns. Moreover, we found that the subset of transcripts that are bound by Srsf3 and undergo AS upon PDGFRα signaling commonly encode regulators of PI3K signaling and/or endosomal trafficking. Finally, we generated a mouse Srsf3 phosphomutant knock-in allele (*Srsf3*^{A7}) through mutagenesis of the seven Akt consensus motifs in Srsf3 and demonstrated that trans-heterozygous *Srsf3*$^{A7/fl}$*;Wnt1-Cre*$^{+/Tg}$ embryos develop severe midline facial clefting.
Conclusions: Taken together, our findings reveal that growth factor-mediated phosphorylation of an RNA-binding protein underlies gene expression regulation necessary for mammalian craniofacial development.

This work is funded by the National Institute of Dental and Craniofacial Research R01DE030864 and F31DE032252.

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

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Category: Graduate Student

Purpose: Craniofacial development is a complex morphogenetic process, disruptions in which result in highly prevalent human birth defects. 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 signaling 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.
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 (GO) 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 MAPK and/or PI3K 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.
Conclusions: This finding indicates that signaling downstream of the various PDGFR dimers targets an overlapping set of transcripts encoding protein serine/threonine kinases for AS. Together, our results demonstrate that AS is the predominant mechanism of gene expression regulation downstream of PDGFR signaling in the facial mesenchyme.

This work is funded by the National Institute of Dental and Craniofacial Research R01DE030864 and R01DE027689.

39TH ANNUAL
RESEARCH DAY

POST DOCS AND RESIDENTS ABSTRACTS

Friday, February 23, 2024

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

DENTAL. INTEGRATED FOR HEALTH.

Alx Function in the Frontonasal Skeleton Revives the Pharyngeal Arch-0 Hypothesis

Jennyfer M. Mitchell, Matt Murry, Colette Dolby, James T. Nichols

Category: Postdocs and residents

In zebrafish larvae, the frontonasal skeleton, or anterior neurocranium, is simplified to two cartilaginous structures, the ethmoid plate and trabeculae, and one parasphenoid bone. We reported that the *alx* gene family of transcription factors is enriched in frontonasal neural crest cells (fNCC) and that Alx3 functions to set chondrocyte differentiation timing programs in the ethmoid plate. fNCC reside anterior to the pharyngeal arch one, which gives rise to the jaw. Through single-cell RNAseq, lineage tracing, and *in situ* hybridization, we found that different subpopulations of fNCC express unique combinations of *alx* genes contributing to frontonasal skeletal patterning. Different combinations of *alx* mutants produce unique anterior neurocranium skeletal phenotypes suggestive of identity transformations. Most dramatically, *alx1;alx3;alx4a* triple mutants are devoid of ethmoid plate, trabeculae, and parasphenoid bone. Instead, cartilage that resembles jaw structures appears in their place. Consistently, pharyngeal arch one specific genes are ectopically expressed in fNCC in these mutants. Moreover, transcriptomic analysis between wild-type and *alx3;alx4a* double mutants shows a gain of pharyngeal arch one identity at the expense of frontonasal identity in a small subpopulation of cells. These data support a model where the *alx* genes confer spatial identity in the developing anterior neurocranium. The proposed anterior neurocranium-to-jaw skeletal transformations 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 establish a new conceptual context for understanding frontonasal dysmorphology and raise fundamental questions regarding how this subpopulation contributes to vertebrates’ frontonasal development.

Acknowledgments to NIDCR grants R00 DE024190-04/S1, R01 DE030448 to JTN, and F32-DE029995 to JMM.

Polymer Coatings to Combat Microbial Growth on 3D Printed Denture-Base Materials

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[2]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, USA
[3]Microbiology, School of Medicine, University of Colorado Anschutz Medical Campus, USA

Purpose

Nearly 41 million Americans wear complete or partial dentures as of 2020, and this number is projected to increase significantly in the coming years. While denture can restore function and confidence in individuals, they often lead to oral complications such as tooth decay, gum disease, yeast infections and pneumonia. The aim of this *in vitro* study is to investigate the potential benefits of incorporating acrylated hydroxy azobenzene (AHA) in 3D printed denture-based resins to inhibit oral infections such as denture stomatitis.

Methods & Materials

The inkjet 3D printing formulation consisted of mono-urethane di(meth)acrylate (MUDMA), benzyl urethane methacrylate (BzUMA), dimer acid diisocyanate ethoxy dimethacrylate (DDI-HEMA), and methacrylic acid (MAA) at 34-, 25-, 19-, and 22-wt% respectively (henceforth referred to as inkjet). Two-parts of this formulation were used to create a redox initiating system with benzoyl peroxide (2wt% or 4wt%) and ethyl-4-(dimethyl amino) benzoate (1wt% or 2wt%) in the presence or absence (control) of 2wt% AHA within 0.8mm x 6.5mm (thickness x diameter, V = 26.55 mm^3) spacer. The polymerization was initiated at room temperature and completed within the Curebox Plus™ (1h at 80°C under ultraviolet light 365nm & 405 nm, 36 Watts). For the *Candida albicans* (*C. albicans*) assay, sterile samples were incubated statically with 1.8×10^5 colony-forming-units (CFU/mL) for 13h at 37°C and 5% CO_2 followed by detaching the biofilm growth on the samples in a water suspension via mechanical force (vortex and sonication) and quantifying CFU. For the *Streptococcus mutans* (*S. mutans*) study, sterile samples were incubated on agar plates spread with 4×10^5 CFU/mL and grown for 24h at 37°C and 5% CO_2. The zone of inhibition radii was measured. Cytocompatibility of the samples were studied via MTT assay using direct contact with human gingival fibroblasts (HGF) cells. Number of samples $n\geq3$.

Results

The AHA-inkjet samples showed a 12-fold reduction in *C. albicans* biofilm growth compared to the control inkjet samples ($p < 0.05$). For the *S. mutans* study, the AHA-inkjet sample inhibited bacterial growth and demonstrated a zone of inhibition (10.2 mm ± 0.30 mm), which were absent in the control samples. Both AHA- and control-inkjet samples were not significantly different ($p > 0.05$) in metabolic activity compared to only HGF cells.

Conclusion

AHA-based 3D printed denture-base materials can significantly inhibit the growth of *C. albicans* and *S. mutans*, thereby giving the potential to remediate risks and complications from removable dental prosthesis. The next phase of the study will focus on quantifying the transient, light-induced mechanical movement of AHA samples (photofluidization effect) from the surface of the dentures to enable biofilm disruption from the surface of the material.

This work is funded by the National Institute of Dental and Craniofacial Research - K25DE027418 and the Gates Grubstake Fund Reference: GGF028-22-03.

Characterization of calcium signaling in cranial neural crest cells during lower jaw development

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

Purpose: The development of the mandible and jaw joint requires the Endothelin 1 (Edn1) – Endothelin receptor type A (Ednra) – Gq/11 signaling axis. This signaling pathway controls fate determination of cranial neural crest cells (NCCs), the bone and cartilage progenitors of the mandible and jaw joint, by regulating the expression of numerous developmental patterning genes. Attenuation of Ednra-Gq/11 signaling results in joint fusions and homeotic transformation of the mandible to maxilla-like structures in humans, mice, and zebrafish. However, the mechanism that connects Gq/11 activity to the regulation of downstream target genes is not fully understood. The Gq/11 family of Gα subunits activates Phospholipase Cβ isoforms, which produces two second messengers, inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 in turn causes a transient increase in the concentration of intracellular Ca^{2+} ions $[Ca^{2+}]_i$. To gain insight into the mechanisms that link calcium signaling to gene regulation, we characterized the *in vivo* dynamics of Gq/11-dependent changes to $[Ca^{2+}]_i$ in cranial NCCs during early embryonic development.

Methods: We visualized changes to $[Ca^{2+}]_i$ in cranial NCCs of zebrafish embryos using two transgenic lines, *sox10:Gal4* and *UAS:GCaMP7a*. GCaMP7a is a fluorescent sensor that increases light emission upon binding Ca^{2+} ions, and the Gal4-UAS system restricts the expression of GCaMP7a to NCCs. Imaging was performed on a spinning disk confocal microscope, and image analysis was performed with Image J and Imaris.

Results: In dual transgenic zebrafish embryos (*sox10:Gal4*;*UAS:GCaMP7a*) at 28 hours post fertilization, we observed oscillations in GCaMP7a fluorescence intensity in NCCs in the first and second pharyngeal arches.

Conclusion: This result indicates that NCCs exhibit $[Ca^{2+}]_i$ flux and that using the *sox10:Gal4*;*UAS:GCaMP7a* dual transgenic line is a feasible approach for monitoring calcium signaling *in vivo*. We are now examining whether the observed $[Ca^{2+}]_i$ flux is dependent on the Edn1-Ednra-Gq/11 signaling axis.

This work is funded by the National Institute of Dental and Craniofacial Research: K99DE032428 to SMK, R01DE029193 and R01DE030448 to JTN, R01DE029091 to DEC.

PDGFRα/β heterodimer activation negatively affects downstream ERK1/2 signaling and cellular proliferation

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Category: Postdoctoral Fellows

Purpose: 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, PDGFRα and PDGFRβ, that dimerize to form PDGFRα homodimers, PDGFR α/βheterodimers and PDGFRβ homodimers. 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.

Methods: We generated and analyzed a cell line stably expressing C-terminal fusions of PDGFRα and PDGFRβ with BiFC fragments corresponding to the N-terminal (V1) and C-terminal (V2) regions of the Venus fluorescent protein, respectively. This approach allowed us to visualize and purify PDGFRα/β heterodimers for the first time.

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 PDGFR α/βheterodimers are rapidly internalized into early endosomes, particularly signaling endosomes, where they dwell for extended lengths of time. We showed that PDGFR α/βheterodimer 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 PDGFR α/βheterodimers. We demonstrated that knockdown of MYO1D leads to retention of PDGFR α/βheterodimers at the plasma membrane, resulting in increased phosphorylation of ERK1/2 and increased cell proliferation. 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.

This work is funded by NIH/NIDCR F32DE032554 (M.B.C.), R01DE027689 (to K.A.F.), K02DE028572 (to K.A.F.).

Fins come and fins go: The *smoothback* zebrafish mutant informs median appendage development.

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Division of Human Genetics, Cincinnati Children's Hospital Medical Center

Category: Postdocs and residents

Paired locomotion appendages, such as limbs, are hypothesized to have co-opted and redeployed the developmental program of unpaired median appendages, such as the dorsal and anal fin. Compared with paired appendages, median appendages remain surprisingly understudied. An early step in fin, as well as limb, formation is aggregation and condensation of undifferentiated mesenchyme at the site of appendage development. These mesenchymal cells form what is known as the fin, or limb, progenitor field, and are derived from somite mesoderm. However, what genes regulate the establishment and positioning of the median fin progenitor field is unknown. Here, we report that a dominant and viable zebrafish mutant we call *smoothback (smb)* does not develop a dorsal fin. In *smb*, the mesenchyme of the dorsal fin field fails to aggregate leading to a complete absence of the dorsal fin, including all skeletal structures. While the dorsal fin is absent, the anal fin is reduced. All other fins are overtly unaffected. Mechanistically, *smb* is caused by a transgenic (*sox10* regulatory sequences driving *Gal4*) insertion into a non-coding region. We found that, this transgene causes broad dysregulation of genes on chromosome 17, where it integrated. Loss of one copy of *pax9*, also located on chromosome 17, fully rescues the *smb* phenotype suggesting that the *smb* phenotype is due to *pax9* misexpression. While heterozygosity of *pax9* rescues *smb*, fish homozygous for the *pax9* mutation develop remarkable ectopic median fins. Further, this fin-gain phenotype in homozygotes is epistatic to the fin-loss phenotype in *smb*. We propose that the transgene insertion generated an ectopic regulatory element that changed *pax9* expression affecting median fin development. Our results give insight into the developmental mechanisms of understudied median fins, which change position, number, and size, or even disappear across evolutionary time.

This work was funded by the National Science Foundation DGE 1938058.

Author: Dr. Paul Rolfes

Category – Residents- Postdoctoral Fellows

Title: Differences in funding approval for standardized Medicaid orthodontic treatment cases submitted to different Medicaid jurisdictions: a controlled prospective survey

Comparison of funding approvals for standardized Medicaid orthodontic treatment cases (Outcomes) submitted (Comparisons and Intervention) to different Medicaid jurisdictions (Participants), a controlled prospective survey (study design).

Introduction:
Medicaid is a federally mandated program, but has little standardization between states in determining orthodontic treatment coverage. This study aims to evaluate if there is consistency in approval rates of standardized orthodontic cases between state Medicaid boards.

Materials and Methods:
Fifty states and Washington DC were surveyed and asked to give Medicaid coverage determinations on six standardized cases, and state prior authorization forms were collected to observe approval criteria.

Results:
Of the nine states that participated in this study, there were statistical differences in the Medicaid prior authorization approval rates for Orthodontic treatment. Of the six cases presented, Colorado approved two. These two cases were also approved by either 40% or 60% of the other states. Cases that Colorado did not approve were approved by 0-30% of the other states. These results demonstrate that Colorado's approval results were statistically different from other states (P=0.0004). Additionally, the patient with an anterior openbite was approved for treatment more often than the other malocclusions presented (p=0.04).

Conclusions:
The states surveyed approved cases at a rate statistically different from Colorado. The case with an anterior open bite was approved at a higher rate than any other case. There are a variety of methods used to determine for prior authorization for orthodontic treatment in the various Medicaid jurisdictions, and consequently there is a variance in what gets approved. Furthermore, it was difficult to get in contact with individuals who were knowledgeable of the approval system and were willing and able to assist the project.

Key words:
Medicaid, Orthodontics, Prior authorization, Handicapping Labiolingual Deviation Index, Salzmann Index

39TH ANNUAL
RESEARCH DAY

LAB STAFF PROFESSIONALS ABSTRACTS

Friday, February 23, 2024

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

DENTAL. INTEGRATED FOR HEALTH.

Title: A Role for *prrx1 G*enes in Median Fin Development

Colette Dolby[1], Raisa Bailon[1], Matthew Murray[1], Lindsey Barske[2] , and James T Nichols[1],
[1]University of Colorado Anschutz Medical Campus, [2]Cincinnati Childrens Hospital

Category: Staff

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. Surprisingly, the more ancestral median fins have been severely understudied in comparison. Median fins arise from paraxial mesoderm, which condenses into fin bud mesenchyme. Previous studies have shown *prrx1a* and *prrx1b* expression in the mesenchyme of pectoral and pelvic limb buds and in the craniofacial skeleton. Using *in situ* hybridization, we show that *prrx1a* and *prrx1b* are both expressed in mesenchyme prior to median fin bud formation and continues expression into all median fin buds. When either *prrx1a* or *prrx1b* is mutated, we observe split median fins and disorganized rays in the caudal fins of adult zebrafish. Alcian blue staining in 13-day post fertilization (dpf) larvae reveal abnormalities in the hypural cartilage in the caudal fins of *prrx1a/b* mutants. Preliminary results observed in gene expression and fin phenotypes indicate a role for *prrx1a/b* in the development of median fin mesenchyme. Uncovering the machinery of median fin development may elucidate general principles of appendage formation and inform congenital limb abnormalities.

This work is funded by NIDCR R01DE029193 and R01DE030448 .

Title: Tuning Inkjet Photopolymer Formulations for Enhanced Properties

Authors: Austyn Salazar, Jeff Stansbury

Category: Lab staff (PRA)

Purpose: This study investigates the use of various urethane based (meth)acrylates in combination with acidic comonomers to achieve low viscosity and high-performance materials for inkjet printing.

Methods: Urethane (meth)acrylates were mixed with different reactive diluents that contained either a urethane or carboxylic acid group. These reactive diluents were added in molar quantities until viscosity dropped below 30mPa*s. Samples for 3-point-bend testing were made using a 365nm curing light at 100mW/cm^2 with post cure at 80°C under 365/405nm light. Conversion was measured using near FT-IR and a Mechanical Testing System (MTS) was used to measure flexural strength, modulus, and toughness.

Results: The formulations listed contain a urethane crosslinker with different reactive diluents either as an acidic comonomer like methacrylic (MAA)/acrylic acid (AA) or a urethane-based diluent like butyl-monourethane monomethacrylate (Butyl-MUMMA) or benzyl-monourethane monomethacrylate (Benzyl-MUMMA). A novel triurethane dimethacrylate (TriUDMA) was used with AA to reduce viscosity substantially to 11.4mPa*s while also reaching an impressive flexural strength of 270MPa and modulus 5.0GPa. Additionally, diurethane dimethacrylate (UDMA) was used in combination with a mono-urethane dimethacrylate (MUDMA) and MAA to reach a similar viscosity of 11.7mPa*s. The mechanical properties are different reaching 190MPa flexural strength and 4.5GPa modulus, and this highlights the tunability of these systems. Other formulations that accomplish low viscosities use MUDMA with diurethane diacrylate (UDA), butyl acrylate (nBA), and isostearyl methacrylate (ISMA) and MUDMA with dimer acid diurethane dimethacrylate (DDI-HEMA) and ethyl hexyl methacrylate. Each formulation is within inkjet range but are tuned to have more flexibility at lower modulus and flexural strength.

Formulation	**Viscosity (mPa*s)**	**Flexural Strength (MPa)**	**Modulus (GPa)**	**Toughness (MPa)**
30:30:30:10 MUDMA:UDA:nBA:ISMA	5.2 (1.2)	107.95 (3.51)	2.47 (0.12)	12.16 (5.63)
36:12:52 MUDMA:DDI-HEMA:EHMA	14.7 (0.7)	52.06 (5.65)	1.19 (0.01)	3.17 (2.43)
TriUDMA + AA (2x)	11.4 (1.4)	271.48 (11.97)	5.03 (0.5)	11.2 (2.5)
50:50 UDMA:MUDMA + MAA	11.7 (0.9)	191.57 (35.76)	4.502 (0.33)	-
50:50 UDMA:BzUMA + MAA	12.5 (0.7)	154.49 (20.79)	4.74 (0.29)	-
MUDMA-C1 + MAA	17 (1.2)	181.34 (21.59)	4.01 (0.22)	7.45 (3.7)
PUTriMA + MAA + AA	27.2 (3.7)	267.77 (33.63)	6.36 (0.25)	8.18 (3.04)

OUTMA + AA (2x)	58.5 (14.80	235.24 (5.55)	5.34 (0.23)	10.38 (3.64)
MUMMA-Butyl	12.9 (3.1)	-	-	-
MUMMA-Benzyl	31 (3.2)	-	-	-
MUDMA	27.9 (2.1)	-	-	-
EHMA	1.8			
MAA	1.4	-	-	-
AA	1.3	-	-	-

Conclusion: This study has successfully created systems that have tunable properties for the use of inkjet multimaterial printing. Viscosity is the major factor for inkjet systems and this data has shown that formulations can be made to achieve a low viscosity (<30mPa*s), while maintaining tunable properties.

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FACULTY ABSTRACTS

Friday, February 23, 2024

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

DENTAL. INTEGRATED FOR HEALTH.

Examining Burnout between Traditional and International Dental Students Training in the U.S.

*Yanira Owens, RDH, MHA, EdD

Purpose: Academic burnout in dental students is a growing area of research. The purpose of this study was to assess if, and to what extent, differences exist in the dimensions of burnout between traditional and international student program (ISP) dental students.

Methods: Traditional and ISP predoctoral dental students who were transitioning from didactic course work to preclinical and clinical education completed an informed consent, a demographic survey, and the Maslach Burnout Inventory-General Survey for Students. Statistical analysis included descriptive statistics, independent samples *t*-test analysis, and Cronbach's alpha analysis.

Results: The response rate from the target population was 58%. A total of 184 surveys were used in the statistical analysis of this study. Statistically significant differences in the burnout dimension of exhaustion, $M = .82$, 95% CI [.41, 1.22], $t\ (108) = 3.97$, $p < .001$, and the burnout dimension of cynicism, $M = .96$, 95% CI [.50, 1.42], $t\ (182) = 4.11$, $p < .001$, existed between traditional and ISP dental students. No statistically significant difference in the burnout dimension of professional efficacy, $M = -.096$, 95% CI [-0.38, 0.18], $t\ (182) = .68$, $p = .50$, was observed between traditional and ISP dental students.

Conclusion: While both ISP and traditional dental students experienced burnout, traditional dental students experienced significantly more burnout along the dimensions of exhaustion and cynicism. The presence of burnout syndrome in both cohorts of students suggests the need to develop program-based strategies that aim to alleviate academic burnout, enhance learning outcomes, and promote the wellbeing of the future dental workforce.