



**IOWA**

College of Dentistry  
and Dental Clinics

**Iowa Section of the  
American Association  
for Dental, Oral, and  
Craniofacial Research**

# **72<sup>nd</sup> ANNUAL MEETING**

Leveraging Science and  
Policies to Improve Oral  
and Craniofacial Health in  
Early Childhood

**2025**



**Dr. Marcelo WB Araujo DDS, MS, PhD**  
Dean & Professor, School of Dental Medicine  
University at Buffalo

## Our Keynote Speaker

**Dr. Marcelo Araujo, DDS, MS, PhD** is the dean of the University at Buffalo School of Dental Medicine and a distinguished professor of periodontology. Prior to his current role, Dr. Araujo was the chief science officer at the American Dental Association (ADA), the CEO of the ADA Science & Research Institute, and the interim CEO of the ADA Foundation. He also has extensive experience in the pharmaceutical industry, making significant contributions to health care products research.

Dr. Araujo holds a PhD in epidemiology and community health and MS from the University at Buffalo. He received his DDS and a certificate in periodontology from Universidade Gama Filho in Brazil.

### **Keynote Address:**

#### ***How Science is Shaping the Future of Dentistry and Healthcare Policy***

Dr. Marcelo Araujo's keynote address outlines the impact of scientific advancements on dentistry. He describes how research transforms clinical practice, public health, and healthcare policy. The lecture emphasizes the importance of evidence-based practices in improving patient outcomes and integrating new scientific knowledge into dental care.

## Our Featured Speakers



**Dr. Aline Petrin**  
MS, PhD

**Dr. Aline Petrin** is an assistant professor in the Iowa Institute of Oral Health Research and Department of Orthodontics. She earned a Masters (2004) and a Ph.D (2007) in Genetics from the Universidade Estadual Paulista, Brazil. Her research focuses on genetic and epigenetic risk factors involved in the etiology of orofacial clefts (OFCs).

### **Featured Address:**

#### ***Epigenomics of Orofacial Cleft***

OFCs are the most common craniofacial birth defects, affecting approximately 1 in 700 births worldwide. OFCs lead to malocclusion, feeding and speech problems, and psychosocial impacts. While both genetic and environmental factors contribute to OFCs, their specific roles in craniofacial morphogenesis remain unclear. Epigenetic factors, like DNA methylation, can increase OFC risk by regulating gene expression and are influenced by environmental exposures. Her featured presentation will describe how the epigenome influences craniofacial birth defects and her team's efforts to translate genomic/epigenomic data into actionable insights for preventive strategies and therapeutic interventions.

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**Dr. Karin Weber-Gasparoni** is a Professor and Chair of the Department of Pediatric Dentistry. She earned her Certificate in Pediatric Dentistry, MS in Dental Public Health, and PhD in Oral Science from the University of Iowa. She co-founded and directs the Infant Oral Health Program at a local WIC program and is board certified by the American Board of Pediatric Dentistry.

### Featured Address:

#### *Birth to Three – Cavity-Free: A Clinical Trial for Preventing Early Childhood Caries*

Early childhood caries (ECC) is a significant public health issue, disproportionately affecting children from minority and low-income families. Her research team is conducting a randomized clinical trial of pregnant mothers until their child is 36 months ago to compare the efficacy of autonomy-supportive videotaped oral health messages to traditional neutral messages. The primary outcome is children’s caries status, with secondary outcomes including changes in oral health behaviors, oral hygiene, dietary habits, and levels of dental plaque and mutans streptococci. This featured presentation outlines preliminary findings and challenges for the ongoing trial.



**Dr. Karin Weber-Gasparoni**  
DDS, MS, PhD





## How Worms Provide Insight into Immune Responses of Immunocompromised Patients

When most people think about worms, fishing or composting come to mind. But Dr. Ransome van der Hoeven, an expert in microbiology and immunology, has made advances in our understanding of innate immune responses by studying the worm, *C. elegans*, a simple nematode.

For higher level organisms, like humans, it can be challenging to separate the immune response of non-immune system cells from the larger immune system, such as white blood cells. This challenge makes it harder for scientists to understand the mechanisms underlying innate immune responses of patients who are immunocompromised, whether from cancer treatment or autoimmune diseases, which it turns makes it harder to treat infections for immunocompromised patients.

Dr. van der Hoeven's work focuses on mitis group streptococci, bacteria that normally live in the mouth and throat but can cause dangerous infections in vulnerable individuals. These bacteria produce hydrogen peroxide ( $H_2O_2$ ), a compound that helps the bacteria defend against other microorganisms while also posing significant risks to human cells.

"Hydrogen peroxide production poses a dual threat," Dr. van der Hoeven explains. "It enables harmful bac-

teria to thrive in competitive microbial environments like the oral cavity, but it also compromises host tissues, leading to inflammation and infection."

By studying these factors in a simple organism without an adaptive immune system, Dr. van der Hoeven's team hopes to gain insight into innate cellular defenses against these disease-causing bacteria.

More particularly, Dr. van der Hoeven and his research team are investigating how basic leucine zipper (bZip) transcription factors, which are a kind of protein that controls numerous cellular processes, can help cells detect and respond to bacterial  $H_2O_2$ .

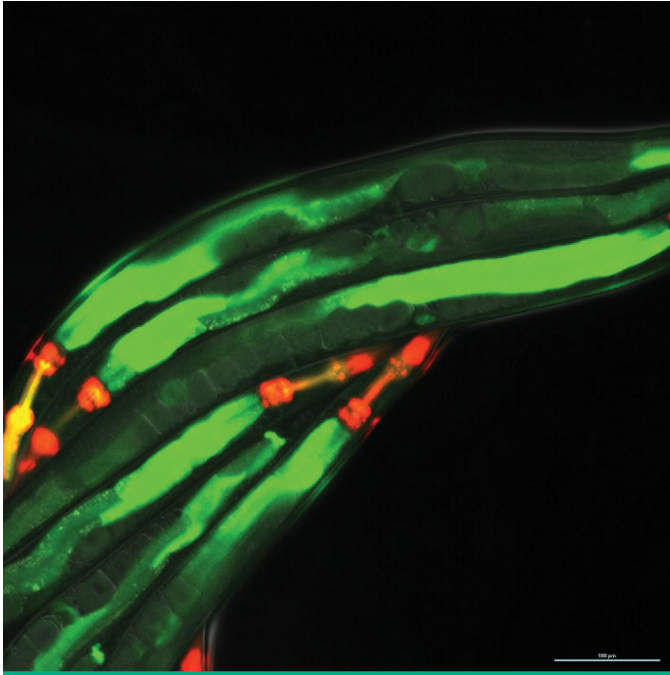
"ZIP-2 and ZIP-10 appear to be key players in managing the oxidative stress caused by bacterial  $H_2O_2$ ," he says, "and our goal is to understand how these factors protect cells, and ultimately the body, from this type of bacterial threat."

Insights gleaned from the *C. elegans* model also hold for human gingival fibroblasts, gum tissue cells directly involved in responding to oral bacterial infections.

"We are using a two-pronged approach to give a clearer picture of the cellular responses to bacterial  $H_2O_2$ ," he explains. "Incorporating human gingival fibroblasts allows us to validate findings from *C. elegans* and extend them to cells more directly involved in oral health."

The implications of this research extend well beyond oral health, potentially leading to therapies that bolster immune defenses against bacterial infections, benefiting not only oral health but also individuals who have an elevated risk for infections due to weakened immune systems.

Dr. van der Hoeven is an associate professor in the Iowa Institute for Oral Health Research and the Department of Periodontics at the University of Iowa College of Dentistry. Since he joined the college in June 2024, he has appreciated the truly collaborative environment.



Worms expressing irg-1 fused to GFP exposed to *S. gordonii*.



Worms expressing ZIP-2 fused to GFP exposed to *S. gordonii*.

“ Our goal is to understand how these factors protect cells, and ultimately the body, from this type of bacterial threat.

– Dr. Ransome van der Hoeven

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He is also deeply committed to mentoring the next generation of scientists and clinicians. He actively incorporates undergraduate students, dental students, and residents in his research, helping them gain first-hand experience in applying basic science to clinical care, as evidenced in his numerous publications with students on topics as diverse as dental education and alternative clinical uses of silver diamine fluoride.

“One of the most fulfilling parts of my role is mentoring those who are passionate about translating discoveries into improved patient care,” he says. “Seeing their growth and commitment to bridging science with practice is incredibly rewarding.”



## Fluoride Skepticism in Focus: Alex Harper's Research on Attitudes about Fluoride Use

As debates surrounding fluoride gain attention across the U.S., a dental student from the University of Iowa is tackling the issue head-on. Alex Harper, a fourth-year dental student, is investigating attitudes about fluoride and tap water. As misinformation erodes trust in science, Harper's research provides timely insights into public perceptions and oral health behaviors.

"There's a lot of pushback on water fluoridation right now," Harper explained. "People hear that fluoride can be toxic, but they often don't realize it's safe in the quantities used in community water fluoridation and has significantly reduced tooth decay."

Motivated by his personal ties to Green Hill, Alabama, Harper surveyed adults in the rural community where he grew up about their knowledge, attitudes, and behaviors related to water fluoridation.

“ People hear that fluoride can be toxic, but they often don't realize it's safe in the quantities used in community water fluoridation and has significantly reduced tooth decay.

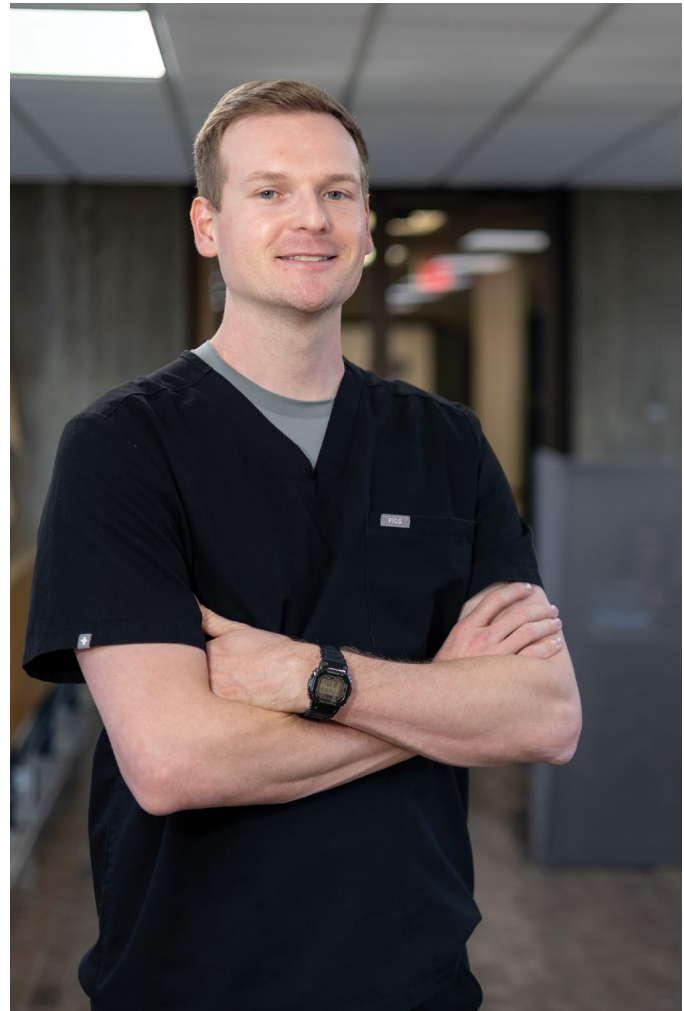
– Alex Harper

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### Revealing Public Perceptions of Fluoride

In his survey conducted in 2022-23, Harper found that 92% of participants believed fluoride prevents cavities, and 84% considered it when choosing toothpaste. Nevertheless, two-thirds reported primarily drinking bottled water at home instead of tap water, although fluoride levels in their tap water did not significantly impact that choice.

"Some results were surprising," Harper said. "We expected people from lower socioeconomic backgrounds to be less accepting of fluoride, but the knowledge, attitudes, and behaviors were consistent across all educational and socioeconomic levels."



Alex Harper is a fourth-year dental student at the College of Dentistry, and wishes to pursue a career in orthodontics.

The findings highlight a disconnect: while residents recognize fluoride's importance, they tend to prefer bottled water, which almost always does not have the optimal level of fluoride. Harper sees this as a call to action for better public education on fluoride's benefits and safety. And it could be that more recent fluoride controversies in the U.S. would shift these findings.

### The Dental Student Research Group and Mentorship

Harper credits much of his success to Dr. Steven Levy, the Wright-Bush-Shreves Endowed Professor of Research at the University of Iowa College of Dentistry.

"Dr. Levy has been a great mentor from day one," Harper said. "When I started, I didn't realize how famous he was, but his knowledge and guidance have been incredible. He's a great mentor, but he hasn't made it easy—he challenges me to think critically."

As a world-renowned leader and mentor in the field, Dr. Levy understands how important it is to understand both the science and public attitudes for public health educational efforts to be successful. “Alex’s research provides a window into the effectiveness and limits of public health messaging about community water fluoridation,” Dr. Levy said.

Harper’s research journey began in his first semester, when he joined the Dental Student Research Group (DSRG). Weekly online classes introduced him to research opportunities and helped him develop his project.

“I didn’t have much research experience during my undergraduate education,” Harper said. “But I knew I wanted to pursue it in dental school, especially since research is so important in specialties like orthodontics, which I hope to pursue.”

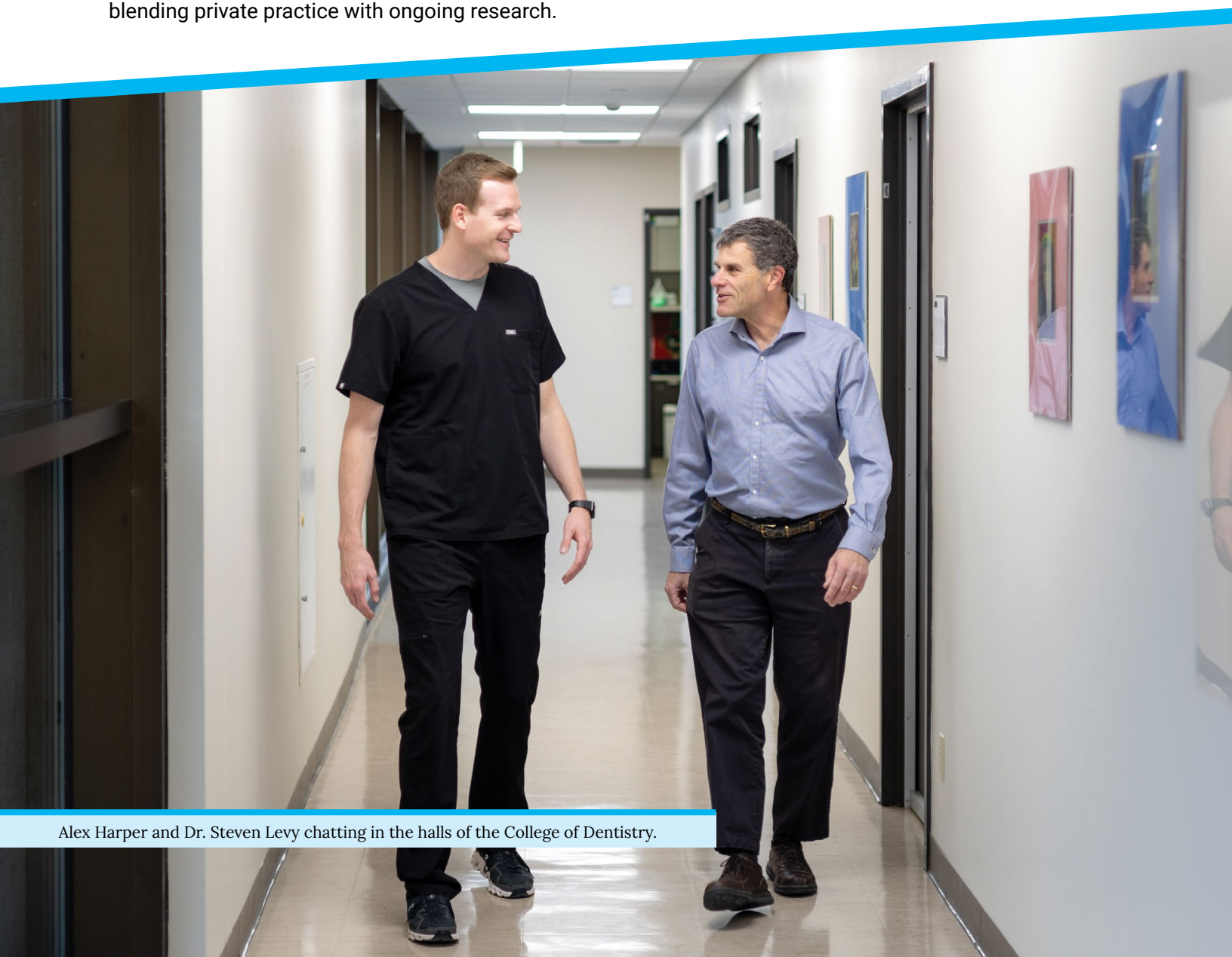
### **Looking Ahead: Research in Practice**

As Harper prepares to graduate, he envisions a career blending private practice with ongoing research.

“In some capacity, I want to stay involved in research,” Harper said. “Understanding how it’s conducted and being able to evaluate the quality of studies will help me make better decisions for my patients. I also hope to move the needle forward by contributing to the field.”

Harper is eager to publish a manuscript based on his findings, ensuring his work informs broader conversations about fluoride and public health. “My educational career might end, but my learning career will never end,” he reflected.

As the dental profession grapples with misinformation, Harper’s work highlights the importance of understanding current knowledge, attitudes, and behaviors for advancing public health. By bridging the gap between science and local communities, his efforts show the power of mentorship and evidence-based practice for the next generation of dental leaders.



Alex Harper and Dr. Steven Levy chatting in the halls of the College of Dentistry.



# Optimizing Your Research: Collaborating with the Division of Biostatistics and Computational Biology

The Division of Biostatistics and Computational Biology at the University of Iowa College of Dentistry is a valuable resource for researchers seeking to enhance the quality and efficiency of their studies so that they are well-designed, data-driven, and powerful.

## How to Get Started

The division strongly encourages researchers to initiate contact as early as possible, ideally before any data is collected for all kinds of projects—pilot studies, student research projects, clinical research, or projects for grants. This proactive approach allows statisticians to contribute to the study design, ensuring that the data gathered is relevant and sufficient to answer the research question and that the project remains on track.

To begin the collaboration process, researchers—including student researchers—can submit a Project Request Form through the division’s online intake form: <https://forms.office.com/r/AvQtjqe9x9>. Alternatively, researchers may reach out directly to the division via email at [dentistry-biostat@iowa.uiowa.edu](mailto:dentistry-biostat@iowa.uiowa.edu) or to specific members of the division for preliminary discussions or to address specific questions.

After completing the intake form or emailing the division, a member of the division will assign a member of the division to the research project based on the PI and statistician’s expertise, research preferences, and workload.

For major projects, such as NIH grants, early engagement with the Division of Biostatistics and Computational Biology is particularly crucial. Larger, complex studies may require years of planning, and early collaboration can help optimize the study design and ensure adequate preparation for grant submission.

*Continued on page 44*



Members of the Division of Biostatistics and Computational Biology at the College of Dentistry. From left to right: Erliang Zeng, Peter Damiano, Shri Vishalini Rajaram, Stephanie Lewis, Wei Shi, Molly Matkovich, Oscar Rysavy, Mahbube Jafari, Jun Cao, J.C. Thomas.



Dear Colleagues,

Thank you for your participation in the 72nd Iowa Section of the American Association for Dental, Oral, and Craniofacial Research held at the University of Iowa on February 11, 2025.

Research and discovery are at the heart of our mission at the University of Iowa College of Dentistry. For decades, Iowa has been at the forefront of dental, oral, and craniofacial research, leading to ground-breaking advancements that have transformed the field. Our commitment to fostering a collaborative research environment has resulted in significant achievements, thanks to the dedication of our talented researchers, both established and emerging.

Today's presentations highlight the innovative work being conducted at our college. I extend my gratitude to our presenters and the planning committee for their invaluable contributions to this event.

We are honored to host Dr. Marcelo W.B. Araujo, DDS, MS, PhD, Dean and Professor at the University of Buffalo School of Dental Medicine, as our keynote speaker. Dr. Araujo is a distinguished academic leader and internationally recognized clinical researcher and epidemiologist. He joined Buffalo in 2023.

Before joining Buffalo, Dr. Araujo served as the chief science officer of the American Dental Association (ADA) and CEO of the ADA Science & Research Institute. He has extensive experience in clinical care, research, and health care business, and has held influential roles in both academia and the pharmaceutical industry.

Dr. Araujo's extensive background and leadership in the field make him an ideal keynote speaker for this event. This day is an opportunity for us to showcase how our research and discovery make an impact on education, service, research, and patient care within our college.

Thank you for being a part of this ongoing work and exciting day.



Clark Stanford, DDS, PhD, MHA  
Dean and Professor, UI College of Dentistry and Dental Clinics

Dear Fellow Researchers:

The Annual Meeting of the local AADOCR is our opportunity to share the expertise and world-class research being conducted at the College of Dentistry and the Iowa Institute for Oral Health Research. Calendar year 2024 was another outstanding year, with almost \$8 million in new research funded by federal, state, and foundation sources, with more pending reviews and notifications ahead. Since 2020, Collegiate investigators have received over \$45 million in external funding. This support provides critical infrastructure, materials, and staffing—allowing us to discover new knowledge, share it with our professional colleagues, and translate it into innovative treatments for our patients and the public.

Student research is a crown jewel of the College and the heart of our local AADOCR annual meeting. We have one of the most active student research programs among any dental school in the world, encompassing predoctoral, masters-level, PhD, and post-doctoral fellows. This year, 44 pre-doctoral students were funded as part of the student research program, with 33 students planning to present at national meetings. Additionally, the NIH renewed funding for our T90/R90 training program, which will continue to support PhD students and postdoctoral researchers.

Our internal research support programs continue to provide funding opportunities for investigators of all career stages. Preliminary research generated through the Seed Grant program and the Clinical/Dental Education Research Initiative Support Program (CRISP), two of the more visible mechanisms, has resulted in multiple external grant applications and millions in federal funding. We have been impressed by our research colleagues' initiative in taking advantage of these internal opportunities to produce cutting-edge research.

Today's events, as highlighted by our three main speakers, demonstrate the wide range of research topics valued at the COD. Dr. Marcelo Araujo, former chief science officer at the ADA and current Dean of the University of Buffalo School of Dental Medicine, brings the perspective of a periodontist, clinical researcher, and epidemiologist. Dr. Karin Weber-Gasparoni is a Department Executive Officer and pediatric dentist with a PhD in oral sciences. As principal investigator for one of the largest NIH grants in the College, her research focuses on behavioral changes and their impact on early-childhood caries. Finally, Dr. Aline Petrin, an assistant professor with a PhD in human genetics, provides insight on the basic science aspects of craniofacial birth defects, epigenetics, and the future of treating these challenging conditions.

Thank you to all those involved in the Collegiate research mission; your contributions are recognized and greatly appreciated. We look forward to celebrating your research efforts and accomplishments today.



Peter C. Damiano, DDS, MPH  
Interim Associate Dean for Research  
College of Dentistry and Dental Clinics



Jeffrey A. Banas, Ph.D  
Director, Graduate Program in Oral Science  
Director, Iowa Institute for Oral Health Research



On behalf of the Iowa Section of the American Association for Dental, Oral, and Craniofacial Research (AADOCR), we extend a warm welcome to you for our 72nd Annual Meeting, themed "Leveraging Science and Policies to Improve Oral and Craniofacial Health in Early Childhood." We look forward to unveiling groundbreaking research discoveries and fostering new collaborative opportunities with our colleagues.

We are honored to feature Dr. Marcelo W.B. Araujo, DDS, MS, PhD, Dean and Professor at the University of Buffalo School of Dental Medicine and former CEO of the ADA Science & Research Institute, as our keynote speaker. Dr. Araujo is a distinguished academic leader and internationally recognized clinical researcher and epidemiologist. Joining him are Iowa's esteemed and accomplished researchers, Dr. Karin Weber-Gasparoni, professor and department executive officer for the Department of Pediatric Dentistry, and Dr. Aline Petrin, assistant professor in the Department of Orthodontics and the Iowa Institute for Oral Health Research. Dr. Weber-Gasparoni conducts clinical research aimed at improving oral health for infants and children from birth until age 3, and Dr. Petrin conducts research on the genetics of cleft lip and palate, a birth defect that affects children around the world. Our lineup of speakers is engaged in cutting-edge work that aims to revolutionize the landscape of oral and craniofacial research across basic science, translational, and clinical domains.

The oral and poster presentations at our event, featuring contributions from numerous graduate students, dental student researchers, and faculty members, are integral to advancing our understanding and practice in the field. These presentations showcase a wide array of innovative research that not only enriches our academic community but also has far-reaching implications for dental and oral health care globally. The University of Iowa's commitment to comprehensive research is exemplified through the diverse topics covered during this annual meeting, ranging from fundamental bench science to clinical applications and policy research.

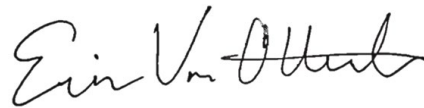
As part of the leadership team for the 2025 Meeting of the Iowa Section of the AADOCR, we warmly invite you to join us for this significant event. We are confident that you will find it as enriching and inspiring as we do. Your participation is crucial to the success of this gathering and to the continued advancement of our field.

Thank you for being a valued member of our vibrant community. We eagerly anticipate the knowledge exchange and networking opportunities that this event will bring, and we look forward to your contributions to this exciting day.

All the best!



Shaoping Zhang, BM, MS, PhD  
President, Iowa Section of the AADOCR  
Assistant Professor  
Department of Periodontics



Eric S. Van Otterloo, Ph.D.  
President-Elect, Iowa Section of the AADOCR  
Assistant Professor  
Iowa Institute for Oral Health Research



Ariene Leme-Kraus, DDS, MSc, PhD  
Vice President, Iowa Section of the AADOCR  
Assistant Professor  
Department of Operative Dentistry



Sheila Britton  
Secretary/Treasurer, Iowa Section of the AADOCR  
Program Coordinator  
Iowa Institute for Oral Health Research

# Program

## Iowa Section of the American Association for Dental, Oral, and Craniofacial Research (AADOCR)

72nd Annual Meeting, Tuesday, February 11, 2025

7:30 a.m.	<b>Breakfast</b> (First Floor Link)
Morning addresses will be presented in the Galagan Auditoria	
8:00 a.m.	<b>Welcome Address</b> Dean Clark Stanford
8:10 a.m.	<b>Welcome Address, and Introduction of Featured Speakers</b> Interim Associate Dean for Research, Dr. Peter Damiano
8:20 a.m.	<b>Featured Speaker</b> Dr. Aline Petrin <i>"Epigenomics of Orofacial Clefts"</i>
8:37 a.m.	<b>Featured Speaker</b> Dr. Karin Weber-Gasparoni <i>"Birth to Three – Cavity-Free: A Clinical Trial for Preventing Early Childhood Caries"</i>
8:55 a.m.	<b>Keynote Speaker Introduction</b> Dr. Shaoping Zhang
9:00 a.m.	<b>Keynote Address</b> Dr. Marcelo Araujo <i>"How Science is Shaping the Future of Dentistry and Healthcare Policy"</i>
9:45 a.m.	<b>Break</b>
10:15 - 11:45 a.m.	<b>Oral Presentations</b> Session 1: Pathogenesis of Inheritance Deformity (Galagan A) Session 2: Clinical Practice & Clinical Practice-Treatment Effect (Galagan B) Session 3 : Behavioral, Epidemiologic and Educational Research (Galagan C) Session 4: Pathogenesis: Microbiology and Immunology (W333) Session 5: Developmental Biology and Genomics (W433) Session 6 : Drug Delivery & Biomaterials in Vitro (N212 Oral-B)
12:00 - 1:00 p.m.	<b>Poster Presentations</b> (Iowa Institute for Oral Health Research, Fourth Floor Link, W220 A/B)



## Oral Session 1: Pathogenesis of Inheritance Deformity

1. **Whole-Genome Sequencing Analyses of Africans with Clefts 2.0: Insights Gained**

**Waheed O. Awotoye**<sup>1</sup>, P.A. Mossey<sup>156</sup>, L.J.J Gowans<sup>127</sup>, M. Eshete<sup>213</sup>, W.L. Adeyemo<sup>128</sup>, A. Alade<sup>205</sup>, E. Zeng<sup>1</sup>, T. Naicker<sup>145</sup>, M.J. Young<sup>49</sup>, N. Ibarra<sup>1</sup>, E.T. Aladenika<sup>1</sup>, M. Olujitan<sup>1</sup>, A.M. Oladayo<sup>1</sup>, A.A. Adeyemo<sup>155</sup>, A. Butali<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA; <sup>49</sup>University of Michigan, Ann Arbor, MI; <sup>127</sup>Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; <sup>128</sup>Department of Oral and Maxillofacial Surgery, University of Lagos, Akoka, Lagos, Nigeria; <sup>145</sup>University of KwaZulu-Natal, Durban, South Africa; <sup>155</sup>National Human Genomic Research Institute, Bethesda, MD; <sup>156</sup>Department of Orthodontics, University of Dundee, Dundee, UK; <sup>205</sup>National Institute of Dental and Craniofacial Research, Bethesda, MD; <sup>213</sup>Addis Ababa University, School of Medicine, Department of Surgery, Addis Ababa, Ethiopia.

**Objective:** Damaging genetic mutations are known to contribute to the risk of the most common craniofacial anomaly, orofacial clefts (OFCs). However, even within families known to carry these mutations, the resulting manifestations can vary from no overt clefting to the presence of different cleft types that vary in severity and laterality. To unravel the genetic etiology of OFCs, which will inform diagnosis and genetic counseling, we conducted the second whole-genome sequencing (WGS) analysis on a large cohort of families with nonsyndromic cleft lip with or without cleft palate (nsCL±P) from Africa, the most genetically diverse human population.

**Methods:** We sequenced the genomes of 148 case-parent trios, each including a child affected by nsCL±P. We screened for pathogenic protein-altering variants using CADD scores = 20 and REVEL scores = 0.5 as well as computed their clinical significance using criteria from the American College of Medical Genetics and Genomics / American Association of Molecular Pathology (ACMG/AMP). Genotype-phenotype analyses were performed using mouse gene expression and human genomics databases to link those pathogenic variants with cleft pathogenesis.

**Results:** Our analyses revealed novel pathogenic protein-altering mutations in several key genes associated with lip and palate development including: *KIF1B*, *QSOX1*, *MEGF6*, *FAP*, *SLC8A1*, *LRP2*, *TTN*, *FLNB*, *COL6A6*, *COL7A1*, *CTNNA2* and *CPO*. Notably, mutations in *LRP2* and *TTN* were previously reported in Africans with cleft following the first WGS analysis, while variants in *CTNNA2* were also reported in the first African genome-wide association study. The presence of these mutations in some unaffected parents suggests a multifaceted causative mechanism underlying OFC phenotypes.

**Conclusion:** This study identifies new pathogenic variants contributing to OFCs, reinforcing the complex causative mechanism for the clinical manifestation of OFCs. These findings enhance our understanding of the mechanisms driving

the most prevalent craniofacial birth defect, paving way for improved diagnosis and genetic counseling strategies.

Supported by: NIDCR DE022378, R01 DE028300

2. **DNA Methylation's Role in Laterality of Non Syndromic Orofacial Clefts**

**Christina E. Spencer**<sup>1</sup>, L. Alves Machado De Paula<sup>1</sup>, F. Qian<sup>1</sup>, W.O. Awotoye<sup>1</sup>, S.M. Dabdoub<sup>1</sup>, A. Butali<sup>1</sup>, M.L. Marazita<sup>59</sup>, L.M. Moreno Uribe<sup>1</sup>, A.L. Petrin<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA; <sup>59</sup>University of Pittsburgh, Pittsburgh, PA

**Objective:** DNA methylation is an epigenetic mechanism that regulates gene expression through the addition of a methyl group to cytosine followed by a guanine (CpG sites). Previously, we studied a family of mirror-twins affected with nonsyndromic clefts of distinct lateralities and their parents; 408 CpG sites with differential methylation were identified.

Results were followed with a small yet independent cohort of individuals with nonsyndromic unilateral right or left clefts which replicated 167 of the 408 CpG sites, including CpG sites near *ZFP57*. To further validate results for CpG cg20228636 in *ZFP57*, we studied a much larger independent cohort using blood and saliva samples to address tissue specific DNA methylation.

**Methods:** Targeted DNA methylation analysis of the CpG site cg20228636 in *ZFP57* was performed after primer optimization and bisulfite conversion followed by qPCR in an independent cohort of 200 DNA samples from blood distributed in 4 equal groups by laterality (i.e. left vs. right) and affection (cleft lip vs cleft lip and palate) and another 200 DNA samples from saliva similarly distributed by laterality and affection. Comparisons of DNA methylation by cleft type and laterality were performed using one-way-ANOVA followed by the post-hoc Tukey-Kramer test, with a statistical significance set at  $p < 0.05$ .

**Results:** For saliva samples, subjects with right cleft lip/cleft lip and palate exhibited significantly higher methylation levels compared to those with left clefts ( $P < 0.05$ ). However, the one-way ANOVA indicated no significant differences in methylation levels when comparing all cleft types. For blood samples, the one-way ANOVA revealed a significant effect of cleft type on methylation ( $P < 0.001$ ). The post-hoc Tukey-Kramer test showed that the mean methylation level in the RCL group was significantly lower than in the LCLP ( $P = 0.006$ ) and RCLP ( $P = 0.002$ ) groups.

**Conclusion:** Results confirmed differential methylation in *ZFP57* affecting laterality of cleft lip and palate phenotypes.

Supported by: NIDCR K01 DE027995, R37 DE08559, U01 DE020057, R01 DE012472, R21 DE016930, R01 DE014667, R01 DE028300; University of Iowa College of Dentistry Student Research Program

### 3. Whole Genome Sequencing of Monozygotic Twins Discordant for Orofacial Clefts



**Austin B. Hinkle**<sup>1</sup>, L. Alves Machado De Paula<sup>1</sup>, H. Keen<sup>1</sup>, M. Chimenti<sup>1</sup>, M.L. Marazita<sup>59</sup>, J. Murray<sup>1</sup>, L.M. Moreno Uribe<sup>1</sup>, A.L. Petrin<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA; <sup>59</sup>University of Pittsburgh, Pittsburgh, PA

**Objective:** Orofacial clefts (OFCs) are a congenital condition that affects approximately 1 in 700 births worldwide, presenting significant challenges for the families and healthcare systems. The multifactorial nature of OFCs include both genetic and environmental influences. Monozygotic twins share most of their genetic makeup, aside from somatic mutations. Therefore, twins discordant for OFCs can elucidate pertinent etiological findings. We present the findings of whole genome sequencing in 13 pairs of monozygotic twins discordant for nonsyndromic OFCs.

**Methods:** Whole genome sequencing was performed at an average coverage of 30x, aligned to GRCh38. We examined rare (MAF < 1%) loss of function (LOF) and/or missense mutations that were present in affected twins and not in their unaffected counterparts. We used bioinformatic tools (Mouse Genomics Informatics Database, gNOMAD, CADD score, VarSome, GeneCards) to identify novel and/or recurrent rare single nucleotide variants, and to assess association with craniofacial phenotypes and pathogenic potential.

**Results:** We found 51 mutations (25 missense, 10 splice site, and 16 frameshift) on the affected members of the 13 pairs, located on 31 unique genes. Top candidate genes include *ACAN*, *ACLY*, *ESX1*, and *MUC5B* among others. *ACAN* is critical for cartilage development and maintenance, which is essential for proper craniofacial morphogenesis and all four genes have been previously associated with clefts.

**Conclusion:** We identified potential nonsyndromic cleft candidate genes based on genomic data of monozygotic twins discordant for OFCs. By unraveling the complex genetic foundation of OFCs susceptibility and discordance, this study aims to pave the way for future research and provide useful information for families and physicians. Ultimately, the data will contribute to the effort to reduce the burden of OFCs and enhance the quality of life for affected individuals and their families.

Supported by: University of Iowa College of Dentistry Student Research Program

### 4. Functional Consequence of IRF6 Variants in van der Woude Syndrome



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**Objective:** Van der Woude syndrome (VWS), the most common syndromic manifestation of orofacial clefts, accounts for 2% of all cleft lip and palate cases. IRF6 mutations explain ~70% of the heritability of this condition, making it crucial to identify high-confidence causal variants to improve risk assessment strategies. Advances in computational biology to validate the functional consequences of variants are essential for developing these strategies. Here, we utilized DDGun3D in predicting the functional effects of variants in IRF6 on VWS.

**Methods:** We conducted Sanger sequencing of 34 families from Nigeria, Ethiopia, Ghana, South Africa, and Puerto Rico to identify variants in the IRF6 gene. This analysis revealed 14 likely causal missense variants, using filtration criteria for SIFT, PolyPhen2, and CADD (>20). Using Force Field X, we optimized AlphaFold3-predicted IRF6 protein structures. We then assessed the stability of the IRF6 protein with these variants using DDGun3D, validating our results with positive controls from previous experimental studies.

**Results:** IRF6 variants in unsolved regions of the protein structure, yielded no predictive scores, however, for variants with available predictions, the results were consistent with previously reported functional data. c.881T>C, known to cause cell rupture, exhibited a high free energy change ( $\Delta\Delta G_{fold} = 4.1$  kcal/mol); indicative of a highly destabilizing effect for the resulting protein structure. Conversely, variants shown to cause cell rescue in experimental studies had low free energy change scores.

**Conclusion:** This study highlighted the effectiveness of using protein structure-based computational approaches for interpreting genetic variants. This method serves as a valuable complement to traditional genetic studies. By combining computational predictions with experimental data, we can gain deeper insights into genetic conditions. However, the success of this approach hinges on our ability to fully understand protein structures; which remains an ongoing area of research.



## 5. Rare Variants Disrupt Craniofacial Enhancer Function Near BMP2 Gene



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**Objective:** Approximately 70% of orofacial clefts are non-syndromic (nsOFC) and influenced by genetic and environmental factors. While common variants explain little of nsOFC heritability, rare and novel variants likely contribute. About 90% of nsOFC-associated variants are in non-coding regions, such as enhancers that regulate gene expression via transcription factor (TF) binding. We hypothesized that rare or novel deleterious variants in craniofacial enhancers may disrupt TF binding and craniofacial gene expression.

**Methods:** We performed whole-genome sequencing on 130 African case-parent trios, using GATK for variant characterization and VarSeq to identify rare (MAF <0.1%) and novel variants in enhancers. We prioritized enhancers overlapping in-vivo validated craniofacial enhancers that contained deleterious variants (CADD score =15) or variants predicted by RegulomeDB to disrupt TF binding. Further prioritization targeted enhancers with rare/novel variants in conserved peak summits. Proximal genes were identified via the UCSC Genome Browser, with their craniofacial roles assessed using MGI, OMIM, and CleftGeneDB. Enhancer activity was evaluated in mice using CRISPR/Cas9 to insert constructs with reference or variant alleles upstream of a lacZ reporter gene at the Hipp11 site.

**Results:** Out of 4,579 putative enhancers, we identified 7,441 rare and novel variants across 2,925 enhancers. Among these, 175 overlapped with validated enhancers, 69 of which were active in facial structures. Twenty-five enhancers contained 46 deleterious variants, with four predicted to disrupt TF binding. Additionally, 12 enhancers harbored rare or novel variants within conserved peak cores. Five enhancers contained multiple variants and were located near cleft-associated genes, including BMP2, BMP4, HS2ST1, FGFR1, and RANBP1. In mice, both a novel variant (g.7516913A>T) and a very rare variant (g.7516992C>T) within an enhancer near BMP2 (chr20:7516128-7517759) resulted in a loss of enhancer activity in facial tissues.

**Conclusion:** Variants in craniofacial enhancer near BMP2 disrupted enhancer function and may affect nearby gene expression leading to nsOFC.

Supported by: NIH R01 DE028300.

## Oral Session 2: Clinical Practice & Clinical Practice-Treatment Effect

### 6. A Novel Method for Evaluating Forces Present During RME Treatment



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**Objective:** Maxillary constriction is a common condition that often leads to maxillary crowding, functional shifts, asymmetric jaw growth and posterior crossbites. The standard orthodontic treatment involves a rapid maxillary expander (RME) to separate the midpalatal suture. Few studies have measured the forces applied to the RME during treatment. This study aims to pilot a novel method for measuring these forces.

**Methods:** Forty-eight patients with either 2 or 4-banded Hyrax-style RMEs were recruited (age: 7-16). Each expander was pre-clinically calibrated before patient delivery. Patients returned daily for 2 jackscrew turns over 12-17 days until clinically sufficient expansion was achieved with upper molars nearing buccal crossbite. The force of each turn was recorded. Photos, diastema width measurement, and intraoral scanning were taken at each visit. After the final day of turning, maxillary occlusal radiographs were taken. The expansion-resisting

forces were estimated based the calibration curve created for each expander.

**Results:** Preliminary analysis confirms that forces range from 0 to 20 lbs, with no decrease in force levels following sutural separation, indicating that resistance to expansion is not solely due to the midpalatal suture alone. Older patients (>12.5 y.o.) exhibited higher overall expansion loads compared to younger patients (<12.5 y.o.). Older patients showed a higher rate of load increase leading up to sutural separation which leveled off after sutural separation, whereas younger patients showed similar rates of load increase before and afterwards. There was no difference in skeletal width between 4-banded or 2-banded hyrax expanders.

**Conclusion:** Older patients presented higher overall forces and greater initial rates of load increase. In most cases, resistance readings continued to rise with each additional day of turns, even after a diastema had formed. Four-banded expanders showed no difference from two-banded expanders in terms of skeletal expansion. Statistical analysis of the outcomes is in progress.

## 7. Longitudinal Immunoprofiling of Adolescents Undergoing Orthodontic Treatment: Preliminary Findings



**Abigail M. Wieland**<sup>1</sup>, S.M. Ganesan<sup>1</sup>, L.M. Moreno Uribe<sup>1</sup>, S.M. Dabdoub<sup>1</sup>, P. Remulla<sup>1</sup>, M. Daily<sup>1</sup>, M.A. Callan<sup>1</sup>, S. Venugopalan<sup>1</sup>, J. Beetner<sup>1</sup>

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**Objective:** Approximately 4.5 million Americans pursue orthodontic treatment annually for esthetic and functional benefits. However, orthodontic therapy poses risks, including compromised oral hygiene, increased susceptibility to caries, gingival inflammation, and recession, all of which are influenced by host-microbial interactions. This study aims to longitudinally evaluate the effects of fixed orthodontic therapy (FOT) on the host immune system by assessing cytokines and immune mediators in saliva and gingival crevicular fluid (GCF).

**Methods:** Twenty-five healthy adolescents undergoing fixed orthodontic treatment were enrolled. Saliva and GCF samples were collected at multiple time points, including baseline, 3 months, 6 months, 12 months, 18 months, debonding, and the 3-month retention check. To date, data analysis has been limited to seven participants with samples available through the retention period. Unstimulated saliva and GCF samples were stored at -80°C. Cytokine and chemokine levels were quantified using multiplex assays, and longitudinal changes were assessed using spline modeling.

**Results:** Fixed orthodontic therapy was associated with significant shifts in cytokines and chemokines across treatment stages, with distinct patterns observed in the salivary and subgingival environments. In saliva, IL-1RA levels significantly decreased toward the end of treatment, while sCD40L levels increased over time. Subgingivally, IL-1A levels were highest at initial stages and decreased as therapy progressed. Chemokines such as CXCL10 and CXCL9 showed increasing trends during treatment. Interestingly, components of the IL-1 family, including IL-1A and IL-1RA, demonstrated consistently high levels in both saliva and GCF.

**Conclusion:** These preliminary findings indicate that FOT induces dynamic and environment-specific immunological changes, reflecting the host's adaptive response to treatment. Continued analysis of the remaining 18 samples will provide a more comprehensive understanding of the immune profile during orthodontic therapy. These results highlight the importance of monitoring immune responses to improve patient outcomes and inform personalized therapeutic strategies.

Supported by: University of Iowa College of Dentistry Department of Orthodontics; NIDCR R03 DE030527

## 8. Nonsurgical Root Canal Retreatment Outcomes Using GentleWave: A Pilot Study



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**Objective:** The GentleWave System (GWS; Sonendo, Laguna Hills, CA) is a multi-sonic irrigation device designed to clean root canal systems through advanced fluid dynamics and acoustic energy. This pilot study aimed to determine the minimum sample size required to compare treatment outcomes of nonsurgical root canal retreatment (NSRTX) using the GWS versus conventional needle irrigation (CNI) based on the change in volume of endodontic lesions as assessed by cone beam computed tomography (CBCT).

**Methods:** Pre- and post-treatment lesion volumes from CBCT images were measured using 3D Slicer software (<https://www.slicer.org/>; Boston, Massachusetts). Lesion volumes between the two groups were compared with a two-sample t-test or a nonparametric Wilcoxon rank-sum test. Within group comparisons of pre- and post-treatment lesion volume were assessed using a paired-sample t-test or the nonparametric Wilcoxon signed-rank test. Data normality was assessed using the Shapiro-Wilk test. All statistical analyses were conducted at a significance level of 0.05.

**Results:** Ten patients (one tooth per patient) were included in this pilot study (5 GWS, 5 CNI). No significant difference was observed between the GWS and CNI groups pre- ( $p=0.323$ ) or post-treatment ( $p=0.060$ ) lesion volume. Within groups, post-treatment lesion size was significantly smaller than pre-treatment lesion size for CNI ( $p = 0.047$ ) and approached significance for the GWS ( $p = 0.063$ ). There was no significant difference in the change in lesion size between groups ( $p = 0.261$ ). To detect a mean difference in change between the GWS and CNI at 80% power, 18 participants per group (total 36) are required.

**Conclusion:** NSRTX appears effective in reducing the size of endodontic lesions with the GWS and CNI. To evaluate the impact of the GWS on the outcome of NSRTX with sufficient power, additional recruitment of participants is ongoing.

Supported by: University of Iowa College of Dentistry Department of Endodontics

## 9. Periotest M Device as a Means of Measuring TAD Stability



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**Objective:** Temporary anchorage devices (TADs), commonly referred to as orthodontic miniscrews, are utilized in treatment as a source of anchorage to support tooth movement. Failure of the TADs due to excessive mobility is common and interrupts orthodontic treatment intervention. The aims of this study are to establish a protocol by which TAD stability can be measured via the Periotest M device, to investigate the validity of such data, and to begin gathering clinical data.

**Methods:** This study contains both an *in vitro* portion and an *in vivo* portion. In-vitro, we aim to assess the intra- and inter-operator variability of Periotest measurements. In-vivo, this is a pilot study to begin collecting clinical data.

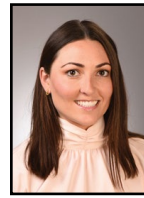
In-vitro, a TAD was placed into a synthetic bone mandible. The mandible was affixed to a benchtop via clamp and a series of 10 measurements were recorded by 11 calibrated operators. The Periotest was held as parallel with the floor as possible. A two-way ANOVA test was used to determine if there was a statistically significant difference within each operator and amongst all operators.

In-vivo, each TAD will be placed following standard clinical protocol and in alignment with the orthodontic treatment plan. After initial placement and at each subsequent visit, each TAD will be measured 3 times by 2 calibrated operators following previously mentioned protocols.

**Results:** Amongst all pooled in-vitro Periotest values by all operators, no significant inter- or intra- operator variability was found. Additionally, the previous phase of this project demonstrated that the Periotest values measured a steady decrease in TAD stability as the TAD was removed one turn at a time.

**Conclusion:** Data collected within this study indicate that the Periotest device may be useful for assessing the stability of TADs in-vivo.

## 10. Trends in Third Molar Extractions Utilizing BigMouth



**Kathryn Jehle**<sup>1</sup>, S. Marshall<sup>1</sup>, K. Shin<sup>1</sup>, L.M. Moreno Uribe<sup>1</sup>

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**Objective:** Wisdom tooth extraction, typically performed by oral and maxillofacial surgeons, is commonly recommended due to the associated risks of tooth impaction. The

American Dental Association estimates that 10 million third molars are removed annually in the U.S., affecting around 5 million patients. Despite the high number of extractions, comprehensive studies quantifying the distribution of third molars in larger populations are lacking. Our preliminary study characterized third molar extraction trends at the University of Iowa College of Dentistry. We now endeavor to gather more data through BigMouth in order to learn about the distribution of third molar extractions nationwide.

**Methods:** We retrieved data from Axium for third molar extractions (2008-2023) in the Department of Oral & Maxillofacial Surgery, resulting in 21,161 patients and 69,171 extracted teeth. Using six CDT codes (e.g., D7140, D7240), we categorized extractions by tooth type, age, gender, and location (maxillary vs. mandibular). To increase the sample size, we utilized BigMouth, allowing for a broader analysis of third molar distribution.

**Results:** The most common extraction code was D7240 (completely bony impaction). Mandibular third molars were more frequently impacted, while maxillary molars were often fully erupted at the time of extraction. Impacted molars were removed at a younger age than erupted ones. Female patients showed a slightly higher impaction rate and tended to undergo extractions at a younger age than males.

**Conclusion:** Mandibular third molars had a higher impaction rate than maxillary molars. We plan to evaluate the larger BigMouth sample to allow reporting of national trends and compare those to what we found at the Iowa College of Dentistry.

## Oral Session 3: Behavioral, Epidemiologic and Educational Research

### 11. Effect of Toothpaste with Charcoal on CAD/CAM Lithium Disilicate Ceramic



**Bayaan Jabr**<sup>1</sup>, C. Jurado<sup>63</sup>, F. Floriani<sup>1</sup>

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**Objective:** CAD/CAM lithium disilicate ceramics are widely chosen for their aesthetic appeal, strength, and durability, making their maintenance and care critical for long-term success. With the rising popularity of whitening toothpastes, understanding how these products interact with lithium disilicate ceramics is crucial. Therefore, this study aims to investigate the effects of toothbrushing and different toothpastes on the surface roughness of CAD/CAM lithium disilicate ceramic dental materials.

**Methods:** The surface roughness of 150 samples of CAD/CAM lithium disilicate samples with different brand pre-crystallized, and fully crystallized were evaluated before and after 18,000 cycles of toothbrushing with distilled water and different Charcoal toothpastes: Crest 3D White Charcoal, Colgate Optic White with Charcoal, Tom's Activated Charcoal, Arm & Hammer Charcoal White, and Conventional toothpaste. Samples were separated into twelve group test and three control group (conventional toothpaste). Each sample were precisely segmented into slices ranging from 1.5 to 2 mm in thickness in a precision cutting machine. The specimens were polished under wet conditions and crystallized according to the manufacturer's instructions. The profilometer were used to measure the (Ra) for each specimen in (µm). Samples microstructural surface were evaluated the surface aspect characterization before and after toothbrushing with Scanning

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Electron Microscope (SEM). The mean values were compared using ANOVA and Post Hoc Tukey's test ( $\alpha = 0.05$ ).

**Results:** Exhibited statistically significant in Ra of lithium disilicate with Charcoal toothpaste compared to conventional toothpaste ( $p < 0.001$ ) and between group pre-crystallized, and fully crystallized ( $p < 0.001$ ). In conclusion, patient and clinician, should be aware

**Conclusion:** In conclusion, patient and clinician, should be aware of roughness Charcoal toothpastes composition, and avoid them with lithium disilicate ceramics.

Supported by: University of Iowa College of Dentistry Student Research Program

## 12. Self-Assessment in Dental Education: Efficacy of a Novel Visual Guide



**Ibtihal A. Sheikhoon**<sup>1</sup>, A. Jain<sup>1</sup>, K. Alzahrani<sup>1</sup>, N. Restrepo-Kennedy<sup>1</sup>, M. Hernandez<sup>1</sup>, T.S. Porto<sup>21</sup>, S. Lewis<sup>1</sup>, J. Kolker<sup>1</sup>

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**Objective:** This study aimed to assess the efficacy of a novel systematic visual guide in enhancing self-assessment and clinical skill development among first-year dental students in preclinical operative dentistry.

**Methods:** A quasi-experimental design was used, involving 70 first-year dental students at the University of Iowa and approved by the IRB [202401434]. Students were randomly assigned to either a control group (no visual guide) or an experimental group (with the visual guide). Both groups completed a Class IV composite restoration exercise and underwent self-assessments and instructor evaluations at three stages: Practice #1, Practice #2, and Practical. Data were analyzed using paired t-tests and Krippendorff's alpha to assess grading reliability and agreement between self-assessments and instructor evaluations.

**Results:** The findings indicated a slight improvement in self-assessment accuracy and instructor evaluation agreement in the experimental group from Practice #1 to Practice #2, but no statistically significant differences were observed. Instructor grader agreement, as measured by Krippendorff's alpha, showed modest improvement after the guide's introduction but remained below acceptable reliability levels.

**Conclusion:** While the visual guide provided structure for self-assessment, its overall impact on improving self-assessment accuracy and agreement with instructor evaluations was limited. Further refinement of the guide and its integration with comprehensive feedback and training is recommended to maximize its educational benefit.

## 13. Treatment Patterns and Characteristics of Rampant Caries: A Retrospective Analysis



**Manal S. Alzahrani**<sup>1</sup>, J.L. Kolker<sup>1</sup>, J.J. Warren<sup>1</sup>, M.M. Hernandez<sup>1</sup>, A. Leme-Kraus<sup>1</sup>

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**Objective:** This retrospective study explores treatment pathways and procedures for patients diagnosed with rampant caries at the University of Iowa College of Dentistry and Dental Clinics (UICOD) from 2010 to 2024. The goal is to identify treatment types and assess treatment completion rates.

**Methods:** The inclusion criteria were: patients aged 16 years or older with at least eight carious lesions, including one or more anterior teeth (teeth 6–11 or 22–27), diagnosed on the same day. Data were extracted from the AxiUm electronic health records using diagnosis codes (C3001, C3003, C3004, C3103, C3104, C3105, C3106, and C4018). Treatment records were followed for 10 or fewer visits to determine the most common pathways or interventions. Descriptive statistics were used to analyze treatment patterns and trends.

**Results:** A total of 7,217 patients were included. Simple extractions were the most common intervention (903 cases), followed by extractions combined with complete upper and lower dentures (355 cases) and multiple extractions alone (238 cases). Conservative treatments, such as composite restorations, were performed in 210 cases, while glass ionomer restorations were documented in 63 cases. Less frequent interventions included amalgam restorations (42 cases) and sedative fillings (36 cases). Many patients did not follow a common treatment pathway.

**Conclusion:** The findings reveal diverse treatment approaches for patients with rampant caries at UICOD. Simple extractions were the predominant treatment, while rehabilitative options like complete dentures and restorative procedures, such as composite restorations, were also utilized. Managing compromised dentition requires balancing functional and aesthetic goals, presenting clinical challenges. These results emphasize the importance of individualized care and preventive strategies to reduce the burden of rampant caries. Further investigation is being carried out to evaluate all procedures and visits to determine treatment completion rates.

## 14. Teledentistry: During Quarantine Portion of Pandemic and One Year Later



**Rima Desai**<sup>1</sup>, J.C. Thomas<sup>1</sup>, P.C. Damiano<sup>1</sup>, J.C. Reynolds<sup>1</sup>

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**Objective:** Teledentistry offers an opportunity for reducing barriers to dental care access. Little is known about the trends in teledentistry use during the quarantine portion of the COVID-19 pandemic and thereafter. This study aimed to 1) examine how teledentistry was used during the first six months of the pandemic and one year later and 2) identify and compare the demographic and professional characteristics

of dentists who were using teledentistry during the first six months of the pandemic and one year later.

**Methods:** Study participants were general dentists in Iowa (n=474) surveyed in August 2021. The dependent variables were 1) teledentistry use during the first six months of the COVID-19 pandemic and 2) teledentistry use one year later. Independent variables included demographic and practice characteristics. Descriptive and bivariate analyses were conducted, and multivariable logistic regression models were generated. A qualitative thematic analysis of how dentists incorporated teledentistry in their practice was conducted.

**Results:** Overall, 18% of dentists utilized teledentistry during the first six months of the pandemic and 8% utilized it approximately one year later. Phone call was the most common modality, and patient triage and consultations for emergencies were the main services provided via teledentistry. In multivariable analyses, dentists that used teledentistry during the first six months were significantly more likely to be women (p=0.026), to be aged 35-44 (p=0.041), and to be accepting new adult patients with Medicaid (p=0.038). No characteristics were associated with teledentistry use approximately one year later.

**Conclusion:** There was a significant decline in the use of teledentistry after the quarantine portion of the COVID-19 pandemic. Practices now have an opportunity to improve access to dental consultations and education with the routine availability of video conferencing post-pandemic. Future research should explore dentist motivations and practice-level factors that may facilitate teledentistry use.

## 15. Interleukin-27 Plays a Protective Role in the Ligature-Induced Periodontitis



**Qiong Ding**<sup>1</sup>, J. Zhang<sup>1</sup>, A. Wang<sup>1</sup>, Y. Qian<sup>1</sup>, S. Zhang<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** Our previous research has shown that in the absence of Type I Interferon signaling mice manifested dramatic bone loss in a ligature-induced periodontitis (LIP) model. Such a severe bone loss pattern was associated with a dampened interleukin-27 response and an exaggerated IL-17 reaction. In this study, we sought to delineate the IL-27-mediated IL-17 response in periodontal disease.

**Methods:** We induced the LIP model in the wild type control mice, mice with disrupted IL-27 signaling (*Wsx*<sup>-/-</sup>), *Act1*<sup>-/-</sup> mice in which the IL-17 pathway was blunted, and the double knockout (dKO) mice (*Wsx*<sup>-/-</sup>/*Act1*<sup>-/-</sup>). The alveolar bone level was compared among different strains of animals. The transcriptional expression of *Il1b*, *Il6*, and *Tnfa* in the ligated gingiva was compared between *Wsx*<sup>-/-</sup> mice to the WT controls. We also isolated CD45+ single immune cells from the ligated gingiva and compared the transcription of several subsets of innate and adaptive immunity lineage markers among different animals.

**Results:** *Wsx*<sup>-/-</sup> mice had more severe bone loss than the WT control mice and the dKO mice in the distal of the first molar, while the dKO mice had similar bone loss to the WT mice. The transcriptional level of *Il1b* was significantly higher in the *Wsx*<sup>-/-</sup> mice than the WT control gingival tissues. In the ligated gingiva, the infiltrated immune cells isolated from the *Wsx*<sup>-/-</sup> mice manifested significantly higher *Il17* and *Inos* transcripts than the WT, while the levels of *Il10* and *Ifng*, which were signature cytokines for Treg and Th1 lineage, respectively, were significantly lower than the WT. Additionally, the transcriptional level of *Il10* and *Ifng* was significantly higher from the CD45+ gingival cells in the dKO mice than the *Wsx*<sup>-/-</sup> samples.

**Conclusion:** The IL-27 pathway plays a protective role in periodontitis by inducing a Treg and Th1 response while inhibiting an IL-17 hyper-activation.

Supported by: NIH R01 DE032307

## Oral Session 4: Pathogenesis: Microbiology and Immunology

### 16. Altered Immune-Metabolomic Axis in Overweight and Obesity



**Jeremy Bobera**<sup>1</sup>, P. Remulla<sup>1</sup>, M. Correia<sup>1</sup>, R. Brown<sup>1</sup>, I. Evans<sup>1</sup>, E. Taylor<sup>1</sup>, L. Hong<sup>1</sup>, S.M. Dabdoub<sup>1</sup>, S.M. Ganesan<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** Obesity affects over one billion people globally and one-third of U.S. adults, with links to increased mortality, diabetes, and cardiovascular disease. Emerging evidence suggests a bidirectional association between obesity and oral health, with individuals with obesity being twice as likely to develop periodontitis.

However, the mechanisms underlying this link remain unclear. The oral mucosa, a key barrier site, involves complex host-microbe interactions, and disruptions in this balance can lead to periodontitis. Therefore, it is essential to identify the end-stage small molecules (metabolites) in this environment, and the host immune response to these molecules. This study aimed to explore the oral metabolome-immune axis in obese patients through a cross-sectional case-control study.

**Methods:** Unstimulated saliva, subgingival plaque, and gingival tissue biopsies (subset of 11 patients) were collected from 40 periodontally healthy obese (OB), overweight (OW), and normal weight healthy (NWH) adults at the University of Iowa College of Dentistry. Subjects were frequency-matched for age, gender, and ethnicity. Gas Chromatography/Mass

Continued >

Spectrometry identified metabolites, which were annotated using the Small Molecule Pathway Database, with enrichment analysis determining significance. Multiplex assays quantified adipokines and cytokines, with statistical significance assessed using MAsLin2. Sparse Partial Least Squares Regression (sPLS) integrated metabolome and immune data.

**Results:** Niche-specific metabolome alterations were observed. Citrulline, Indoleacrylate, Proline, and Isoleucine were enriched in NWH, while metabolites such as adenine, beta-hydroxybutyrate, niacin, and glucose were elevated in OB. Proinflammatory mediators (e.g., MCP-1, TNF $\alpha$ , IL-1b, IL-2, EGF) were significantly enriched in OB and correlated with metabolites linked to periodontitis.

**Conclusion:** This study reveals distinct oral metabolome and immune alterations in obese patients, even without clinical periodontal disease. These findings highlight the need for personalized treatment strategies for this population

Supported by: NIDCR R03 DE030527; University of Iowa College of Dentistry Seed Grant

## 17. Exploring the Oral Virome in Metabolic Syndrome and Obesity



**Mahbube Jafari**<sup>1</sup>, P. Remulla<sup>1</sup>, S. Vazana<sup>1</sup>, J. Fiedorowicz<sup>196</sup>, H. Laroche<sup>52</sup>, M. Correia<sup>1</sup>, S.M. Ganesan<sup>1</sup>, S.M. Dabdoub<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA; <sup>52</sup>University of Missouri, Kansas City, Kansas City, MO; <sup>196</sup>University of Ottawa, Ottawa, ON

**Objective:** More than one-third of the US adult population suffer from Metabolic Syndrome (MetS) a cluster of risk factors including central obesity, blood pressure elevation, low HDL cholesterol, high triglycerides, and hyperglycemia/insulin resistance. MetS is linked to increased risk for Type 2 Diabetes and Cardiovascular Disease, and emerging evidence for increasing severity of periodontitis, a microbially-induced inflammatory disease. While ongoing work is analyzing microbial populations in this system, no work has examined the viral component of the oral microenvironment and the impact of the increased inflammation in MetS. Thus, this study aimed to explore the role of the oral virome in individuals with MetS and obesity.

**Methods:** Fifty-three subjects were recruited in three groups: MetS patients, Metabolically Healthy Obese (MHO) individuals, and Normal Weight Healthy (NWH) controls. Metagenomic sequencing of subgingival plaque was filtered for quality and human reads. Reads were assembled with MEGAHIT, viral contigs and taxonomy predicted with geNomad, and CheckV used to assess quality. Quantitative analysis and visualization were performed with QIIME2, phyloToAST, and MaAsLin2.

**Results:** A total of 7139 predicted viral genomes were recovered: 120 complete genomes and 162 High-Quality. Distinct differences were seen in virome composition between MetS, MHO, and NWH groups. Beta diversity (Aitchison, Bray-Curtis) showed significant clustering between NWH and MetS/MHO ( $p < 0.001$ , PERMANOVA). MaAsLin2 indicated over 300 viruses to be differentially abundant between all three groups. Finally, a GradientBoosting classifier (1000 trees, 10-fold CV) was able to predict class membership with an average ROC AUC of 0.86.

**Conclusion:** The findings suggest that alterations in the oral virome may play a significant role in the metabolic and inflammatory dysregulation observed in MetS patients and may provide potential targets for therapeutic interventions in MetS and related conditions.

Supported by: NIDCR T90 DE023520, R03 DE030527, R03 DE033993

## 18. Strain-Level Metagenomic Insights into Oral Microbiota in Metabolic Syndrome



**Priscilla Remulla**<sup>1</sup>, S.M. Dabdoub<sup>1</sup>, S. Vazana<sup>1</sup>, J. Fiedorowicz<sup>196</sup>, H. Laroche<sup>52</sup>, M. Correia<sup>1</sup>, S.M. Ganesan<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA; <sup>52</sup>University of Missouri, Kansas City, Kansas City, MO; <sup>196</sup>University of Ottawa, Ottawa, ON

**Objective:** Metabolic Syndrome (MetS) affects over one-third of U.S. adults and is associated with a heightened risk for Type 2 Diabetes, cardiovascular diseases, and periodontitis. MetS patients are twice as likely to develop periodontitis, but the mechanisms behind this link remain unclear. One possible explanation is the disruption of the delicate balance between the oral microbiome and the host immune system. Strain-level analysis is crucial since different bacterial strains, even within the same species, can have distinct functions—some driving inflammation, while others contribute to maintaining homeostasis. This level of granularity is essential to identifying microbial drivers specific to disease, which are often overlooked in broader taxonomic approaches. We aimed to perform a detailed metagenomic strain-level analysis of the subgingival microbiome in MetS patients without periodontitis, in order to uncover microbial signatures that may mediate the connection between metabolic and oral health.

**Methods:** We recruited 200 periodontally healthy adults, divided into three groups: MetS, metabolically benign obesity (MBO), and normal-weight controls (NWH). Subgingival plaque samples were collected, microbial DNA was extracted, and genomic sequencing was performed using the BGI DNBSEQ platform. Strain-level profiles were mapped with Woltka, and differentially abundant strains were identified using PERMANOVA and MaAsLin2.

**Results:** Principal component analysis revealed significant clustering among the groups based on their metabolic status ( $p=0.0001$ , ANOVA). Specifically, virulent strains of *Porphyromonas gingivalis* (W83, WW2903) and *Selenomonas* (F0473, W7667) demonstrated a 4-5 log-fold increase in MetS and MBO groups compared to NWH controls (FDR adj  $p$ -value  $< 0.0002$ ).

**Conclusion:** Our strain-level metagenomic approach is the first to reveal that MetS and MBO patients, despite lacking clinical periodontitis, possess a subgingival microbial environment that closely resembles the periodontal disease state. This highlights an “at-risk-for-harm” microbiome that may predispose these patients to oral health issues in the future and needs to be validated longitudinally in diverse populations.

Supported by: NIDCR R03 DE030527; University of Iowa College of Dentistry Seed Grant



## 19. Metformin Induced Survival of *Caenorhabditis elegans* on *Streptococcus gordonii*



**Balasubramaniam Boopathi**<sup>1</sup>, A. Al Hatem<sup>1,14</sup>, A. Villhauer<sup>1</sup>, R. van der Hoeven<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA; <sup>14</sup>University of Texas Health Science Center at Houston School of Dentistry, Houston, TX

**Objective:** Mitis group streptococci are opportunistic pathogens that reside in the oral cavity and generate hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as major contributor to their pathogenesis. Previously, we established that mitis group mediates killing of the nematode *Caenorhabditis elegans* via H<sub>2</sub>O<sub>2</sub>. Further, we have shown pretreatment of worms with metformin mediates protection from streptococcal-derived H<sub>2</sub>O<sub>2</sub> and is attributed to the activation of the oxidative stress response transcription factor SKN-1/NRF2. Using worms expressing SKN-1 fused to green fluorescent protein, we have shown the p38 MAPK pathway and the transcription mediator complex protein MDT-15 are required for the activation of SKN-1 by metformin. Based on the data, we will further evaluate the activation of SKN-1 by p38 MAPK and MDT-15.

**Methods:** To determine the activation of the PMK-1 (p38) in response to metformin and the dependence of components of the p38 MAPK pathway NSY-1 and SEK-1, we compared the level of phosphorylation of PMK-1 in wild-type (WT) and *nsy-1* and *sek-1* worms pretreated in the presence or absence of metformin by Western blot. Furthermore, the levels of phosphorylation of NSY-1 were compared in the presence or absence of metformin in WT and *nsy-1* worms. Lastly, we compared the levels of phosphorylation of PMK-1 in WT and *mdt-15* worms as described above.

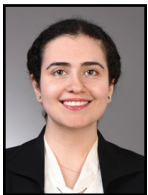
**Results:** A significant increase in the levels of phosphorylation of PMK-1 was observed in the presence of metformin which was dependent on *nsy-1* and *sek-1*. Furthermore, the levels of phosphorylation of NSY-1 were dependent on the presence of metformin. However, a significant increase of phosphorylation of PMK-1 was observed in *mdt-15* compared to WT in the presence or absence of metformin.

**Conclusion:** Based on the data we have established that metformin mediated activation of SKN-1 is dependent on the p38 MAPK pathway. Conversely, SKN-1 activation by MDT-15 is independent of PMK-1.

Supported by: NIH R01 AI158429; University of Iowa Faculty Start Up Fund

## Oral Session 5: Developmental Biology and Genomics

### 21. Functional Assessment of an Oculo-Auriculo-Vertebral Spectrum Associated Variant in Zebrafish



**Saba Manafi**<sup>1</sup>, L.A. Machado De Paula<sup>1</sup>, K. Kannenberg<sup>1</sup>, L.M. Moreno Uribe<sup>1</sup>, S. Rengasamy Venugopalan<sup>33</sup>, A.L. Petrin<sup>1</sup>, E. Van Otterloo<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA; <sup>33</sup>Tufts University, Medford, MA

**Objective:** Oculo-auriculo-vertebral spectrum (OAVS) is a prevalent craniofacial disorder affecting approximately 1 in 3500 births, characterized by a range of craniofacial differences. Despite its frequency, the genetic mechanisms behind OAVS remain unclear. Whole-genome sequencing of a three-generational family with OAVS identified the *PDGFRA* gene as having the highest likelihood of pathogenic involvement. This study aimed to investigate the role of *PDGFRA* in OAVS by using zebrafish as a model. The specific objectives were to perform loss-of-function assays for *PDGFRA*, assess the resulting phenotypic changes, and determine if introducing human *PDGFRA* variant mRNA can rescue or alter these developmental defects.

**Methods:** Zebrafish *pdgfra* function was disrupted using morpholino oligonucleotide (MO) knockdown or a genetic knockout approach. For MO injected embryos, Western blot analysis was used to verify reduced *pdgfra* levels. Following MO or genetic disruption of *pdgfra*, embryos were examined for craniofacial abnormalities using alcian blue staining to highlight skeletal structures. Unaffected or OAVS-associated human *PDGFRA* variant mRNA will be introduced to evaluate its potential to restore normal development in *pdgfra*-deficient

embryos. Phenotypic assessments will be conducted between groups.

**Results:** Western blot analysis confirmed reduced *pdgfra* protein in MO-injected embryos. Genetic or MO-induced disruption of *pdgfra* in zebrafish resulted in craniofacial abnormalities, some overlapping those observed in OAVS, including macrostomia. Ongoing studies are assessing if the introduction of human *PDGFRA* variant mRNA modifies the developmental defects induced by *PDGFRA* disruption.

**Conclusion:** This study supports previous work identifying a critical role of *PDGFRA* in zebrafish craniofacial development, further linking variants identified in this gene to OAVS pathology. Further, mRNA based experiments may determine the exact pathogenicity of the OAVS variant identified. These findings contribute to a better understanding of OAVS and may inform future genetic counseling and therapeutic approaches.

Supported by: University of Iowa College of Dentistry Student Research Program

### 22. Functional Shifts of Toxin-Related Microbial Genes in Dental Caries



**Shri Vishalini Rajaram**<sup>1</sup>, P. Singh<sup>1</sup>, E. Zeng<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** Dental caries affects 3.5 billion adults and 573 million children globally, with oral microbiota playing a central role in development. The molecular mechanisms underlying microbial genes and pathway alterations in healthy, caries-affected, and treated (fluoride and arginine) conditions are not fully elucidated. This study aimed

Continued >

to delineate toxin-related microbial gene expression profiles within the oral microbiome and to determine how these profiles are modulated by caries and therapeutic interventions using high-throughput metatranscriptomics.

**Methods:** We used two RNA-seq-based metatranscriptomic datasets: the Carda-Diéguez et al. (2022) (Carda dataset) and the Ev et al. (2023) (Daniela dataset). These datasets included supragingival plaque samples from caries-free, caries-affected, fluoride-treated, and fluoride-arginine-treated individuals. We processed the datasets using the Multi-Organism, Multi-Omics Microbiome Analysis Pipeline (MOMO-MAP) to identify functional gene clusters, which were then cross-referenced to UniRef90 toxin clusters to discover toxin-related genes. We performed differential expression analysis using ANCOM-BC and functional profiling by mapping sequencing reads to InterProScan, TADB3.0 (bacterial toxin-antitoxin database), and VFDB (Virulence factor database) and by machine learning-based gene ranking to identify key clusters using Bootstrap Ensemble Attribute Ranking (BEAR) was employed.

**Results:** The overlap between UniRef90 clusters and the MOMO-MAP identified 563 clusters unique to the Carda dataset, 174 unique to the Daniela dataset, and 452 shared by both. While no significant differential expression was observed in the dataset-specific clusters, the Type II toxin-antitoxin system - VapC family toxin showed significant differential expressions across healthy, caries, and intervention groups. Furthermore, functional profiles and BEAR analysis highlighted several toxin-antitoxin-related genes, including antitoxin components from the ribbon-helix-helix domain, the YwqK family antitoxin, and the addiction module toxin ReE (Type II system).

**Conclusion:** Our findings advance the understanding of the oral microbiome's involvement in toxin-antitoxin systems and their potential modulation in dental caries. Identifying key toxin-antitoxin genes, particularly those involved in microbial stress responses and survival, suggests a novel link between these systems and caries pathogenesis, which warrants further experimental validation.

Supported by: James S. and Janice I. Wefel Memorial Research Award

### 23. Loss of MEMO1 Results in Hypomineralized Enamel



**Samantha D. Wuebker**<sup>1</sup>, E. Van Otterloo<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** Amelogenesis imperfecta (AI), which affects 1 in 15,000 infants, is a rare genetic disorder characterized by defective enamel formation. Enamel is discolored, pitted, and susceptible to wear, and if untreated, results in tooth pain, sensitivity, increased susceptibility to caries. Notably, 50% of AI cases lack an identified genetic cause—with the genes identified largely represented by enamel matrix proteins—highlighting the need to identify novel genes and cellular processes involved in enamel formation and AI pathogenesis. In previous studies, we identified Mediator of Cell Motility 1 (MEMO1) as a crucial factor in enamel integrity. Loss of Memo1 in ameloblasts of mice resulted in an AI-like phenotype, with enamel prone to wear and breakage.

These findings were observed in aged mice, but the early developmental role of MEMO1 in ameloblasts remained unclear. Based on MEMO1's expression in ameloblasts during embryonic development, we hypothesized that the enamel defects in aged mice were a consequence of a much earlier, developmental and perinatal defect.

**Methods:** To test this, we examined ameloblast and enamel progression from the earliest stages of their development. Using our *in vivo* approach, we conditionally deleted MEMO1 from ameloblasts and analyzed the phenotype of the mutants compared to controls using micro-computed tomography, scanning electron microscopy, and histology.

**Results:** Conditional deletion of Memo1 in ameloblasts, *in vivo*, resulted in significant enamel volume and mineralization defects, relative to controls, and closely resembled defects seen in human AI.

**Conclusion:** Ongoing investigations aim to uncover the mechanisms by which MEMO1 regulates enamel mineralization, potentially improving diagnostic strategies and risk assessment for enamel-related mineralization defects.

Supported by: NIDCR R00 DE026823, T90 DE023520, F31 DE034609; University of Iowa College of Dentistry Seed Grant

### 24. Novel Prognostic Gene Pairs Derived from Multi-Omics Approaches in OSCC



**Jihyun Kim**<sup>1</sup>, S. Rajaram<sup>1</sup>, P. Nguyen<sup>1</sup>, A. Peng<sup>223</sup>, X.J. Xie<sup>1</sup>, E. Zeng<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA; <sup>223</sup>University of Nebraska Medical Center, Omaha, NE

**Objective:** Many OSCC cases are diagnosed at advanced stages, complicating treatment and lowering survival rates, while early detection significantly improves prognosis. This study aims to investigate tumor biology using a multi-omics approach to identify novel prognostic gene pairs that can enhance early detection, improve prognostic accuracy, and support OSCC treatment monitoring.

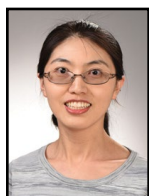
**Methods:** We obtained gene expression (GE) and methylation (ME) data for head and neck squamous cell carcinoma (HNSC) from TCGA GDC portal. Using ICD-10 codes, we extracted oral-specific samples (OSCC, n=337). An ensemble feature selection method was applied to identify differentially expressed genes and differentially methylated CpG sites (Feature-Set 1). Survival analysis on individual genes and CpG sites highlighted those associated with varying survival rates (Feature-Set 2). To enhance prognostic predictions, we conducted pairwise survival analysis separately on both datasets (GE and ME) to identify significant gene pairs/CpG sites from each feature set. Subsequently, we annotated the CpG sites to their corresponding genes. We then analyzed SNPs related to each gene pair in different expression/methylation groups to assess their association with survival outcomes. Finally, network analysis on gene pair sets was performed using two datasets from STRING DB.

**Results:** The ensemble feature selection identified 15/1 genes/CpG sites (Feature-Set 1). Survival analysis revealed 3420/14123 significant (p-value < 0.05) genes/CpG sites (Feature-Set 2). Survival analysis of gene pairs/CpG site pairs showed their combined predictive power surpassed that

of any single one. An intersection of significant gene pairs and CpG site pairs (annotated to genes) revealed 6 key genes, including DKK1, CPNE8, SYT1, SMAD3, TNXB and DIO3. SNPs mapped to significant gene pairs exhibited varying patterns in patient subgroups stratified by high or low gene expression/methylation levels of each gene pair. By integrating the gene expression and methylation protein networks, we identified 704 proteins shared between the two networks.

**Conclusion:** This OSCC multi-omics analysis identified critical genes with strong prognostic potential, with gene pairs offering enhanced predictive power.

## 25. Cross-Repression of *Pitx2* and *Tfap2a/b* Regulates Early Mandibular Lineage Specification



**Fan Shao**<sup>1</sup>, A. Phan<sup>1</sup>, J. Thompson<sup>1</sup>, S. Venugopalan<sup>1</sup>, B.A. Amendt<sup>1</sup>, E. Van Otterloo<sup>1</sup>, H. Cao<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** The establishment of dental epithelial cell lineage within the mandibular epithelium along the dorsal-ventral axis during dental lamina formation remains a critical unanswered question.

**Methods:** To identify potential genes and transcriptional regulatory networks involved in specifying dental epithelial cells within the continuous naive mandibular epithelium, we performed RNA-seq to establish mandibular epithelial gene expression patterns along the dorsal-ventral axis during dental

lamina formation (E11.5). We also examined the functions of these domain specific transcription factors (TFs) in *Pitx2* general knockout (*Pitx2* KOs) and *Tfap2a/Tfap2b* ectodermal double knockout (*Tfap2a/b* EDKO) mouse models. Additionally, we performed CUT&RUN-seq to identify the downstream targets of *Pitx2* and *Tfap2a/b* respectively.

**Results:** We comprehensively identified TFs and signaling pathways differentially expressed along dorsal-ventral axis of mandibular epithelium. With two knockout mouse models: *Pitx2* KOs (dental lamina-specific TF) and *Tfap2a; Tfap2b* EDKOs (ventral surface specific TFs), we found *Tfap2a* and *Tfap2b* establish the ventral epithelium identity by upregulating multiple WNT ligands, subsequently activating WNT pathway activity in ventral mesenchyme. Similarly, *Pitx2* promotes expression of FGF ligands in dental lamina. CUT&RUN-seq reveal distinct set of downstream targets of *Pitx2* and *Tfap2a/b*. In addition, we identified a cross-repression mechanism between *Pitx2* and *Tfap2a/b* both *in vitro* and *in vivo*, suggesting functional antagonism between these domain-specific TFs. Moreover, we found the cross-repression between *Pitx2* and *Tfap2a/b* regulates the underlying mesenchymal SHH and WNT pathway activity, which interface at the site of wild-type incisor formation and define the location of the ectopic incisor.

**Conclusion:** These results uncover a previously unknown molecular mechanism involving cross-repression of domain specific TFs, *Pitx2* and *Tfap2a/b*, in establishing and localizing dental epithelial cell lineage along dorsal-ventral axis of the mouse mandible.

Supported by: NIDCR R01 DE033009

## Oral Session 6: Drug Delivery & Biomaterials *in Vitro*

### 26. Human Adipose Derived Stem Cell Membranes for Bone Regeneration



**Anna Hedges**<sup>1</sup>, J. Hu<sup>1</sup>, Z. Wang<sup>1</sup>, L. Zhong<sup>1</sup>, H. Sun<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Background:** Obtaining adipose tissue is a better alternative to harvesting stem cells versus getting the stem cells from bone marrow. Research has shown bone marrow stem cell membranes have some promise in tissue regeneration through coating micro/nanoparticles or macroporous scaffolds. Stem cell membranes from adipose cells have yet to be investigated for bone tissue engineering and drug delivery.

**Objective:** Study human adipose-derived stem cells (hADSCs) membranes for drug loading capacity and osteogenic potential.

**Methods:** The hADSCs (from Lonza) were expanded and cultured for membrane vesicle isolation, and their size and zeta potential were characterized by using Dynamic Light Scattering (DLS). The membrane vesicles were labeled with PKH67. Human bone marrow stem cells (hBMSC, from Lonza) were treated with cell membranes and imaged using confocal

microscopy. The membranes were loaded with osteogenic fludrocortisone acetate (FA) through sonication and collected by high-speed centrifuge. After seven days of incubation of membrane +/-FA, alkaline phosphatase (ALP), an early marker of osteogenic differentiation, activity of hBMSCs was quantified following the kit manual. Each sample was run in triplicate.

**Results:** The average size of membrane vesicles was 550.75nm, and the zeta potential was -5.93 mV. hBMSC can effectively take up PKH67-labeled hADSC membranes. Our preliminary test suggested that the hADSC membrane vesicles can barely affect the ALP activity of hBMSCs. Interestingly, we found hADSC membrane vesicles have the capacity to load the osteogenic FA and significantly increases ALP activity compared to control groups after 7 days of culture.

**Conclusion:** These results indicate that hADSC membrane vesicles have the potential for bone tissue engineering, but further studies are needed. Collecting hADSC is a less invasive procedure than collecting hBMSC. This finding indicates that hADSC membranes could be used as an alternative new tool for regenerative medicine.

Supported by: University of Iowa College of Dentistry Student Research Program; University of Iowa Faculty Start-Up Funds



## 27. Pilot Testing of RNA Based Therapeutic Approaches for Bone Regeneration



**An.-Vi Phan**<sup>1</sup>, F. Shao<sup>1</sup>, H. Cao<sup>1</sup>, Z. Fang<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** Currently, BMP2 is delivered via bone graft and must be delivered in high concentrations to be effective. However, these high concentrations have been shown to result in adverse side effects. The objective of this

pilot study is to test the stability and translation efficiency of modified RNA and circular RNA to deliver the therapeutic effects of BMP2 while alleviating negative side effects.

**Methods:** Human BMP2 gene was cloned into circular RNA and modified RNA systems that were recently developed to enhance protein production. The circular RNA and modified RNA encoding GFP and BMP2 were transfected into 293T cells and 3T3-E1 cells and were used to measure *in vitro* stability and translation. GFP protein production was visually inspected using fluorescent microscopy, qPCR was used to assess BMP2 circular RNA and modified RNA stability *in vitro*, and ALP was used to assess protein production and cell differentiation progression.

**Results:** Fluorescence measurements of EGFP showed prolonged expression of EGFP over one week, with the highest levels of expression on Day 2 for transfected circular RNA and modified RNA. qPCR results showed minimal degradation of BMP2 of circular RNA and modified RNA over a four-day period, with the highest levels of expression on Day 2.

**Conclusion:** This pilot study successfully cloned human BMP2 into circular RNA and modified RNA production plasmids and showed that circular RNA and modified RNA could be efficiently translated *in vitro*. The cloned BMP2 will be useful in future studies to further investigate RNA expression through additional qPCR and ALP testing. Additional optimization of the transfection protocol could further improve the stability and translation efficiency of BMP2. Long term goals of this study are to develop RNA based therapeutic approaches for bone regeneration *in vivo*.

Supported by: NIDCR R01 DE033009; University of Iowa College of Dentistry Seed Grant

## 28. Lactate Modulates Inflammatory Microenvironment and Bone Metabolism for Bone Regeneration



**Linna Zhong**<sup>1</sup>, Z. Wang<sup>1</sup>, H. Sun<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** Lactate, long treated as a detrimental metabolite, has recently been recognized for its potential anabolic contribution through immunomodulation in some chronic diseases. Interestingly, recent

study indicates patients with osteoporosis exhibit relatively low lactate levels. However, its role in directly regulating bone metabolism remains largely elusive. This study aims to investigate the role of lactate in modulating local inflammation

and osteoblastic/osteoclastic differentiation, and thereby to develop a novel bioengineering approach for bone regeneration.

**Methods:** Macrophage J774A.1 cells were stimulated with LPS to induce an M1-like phenotype and treated with varying concentrations of lactate. After 24 hours, the cells were observed for morphology changes, and expressions of pro-inflammatory factors, including tumor necrosis factor (TNF- $\alpha$ ) and IL-6, were detected using ELISA. Pre-osteoblast MC3T3-E1 cells and mouse primary bone marrow derived stem cells (mBMSC) were treated with lactate for osteogenic differentiation. Alkaline phosphatase (ALP) activity and Alizarin Red staining were quantified on day 7 and 21 days of culture, respectively. Pre-osteoclasts Raw 264.7 cells were incubated with lactate and RANKL, and TRAP staining was performed to evaluate lactate's effect on osteoclastogenesis.

**Results:** We found that lactate, as a main metabolite of glycolysis, demonstrated excellent biocompatibility. ELSIA data indicated that lactate significantly and dose-dependently mitigate the expression of TNF- $\alpha$  and IL-6 in J774A.1 cells. Remarkably, ALP activity was increased significantly both for MC3T3-E1 and mBMSC cells. Alizarin Red staining showed that lactate treatment enhanced the mineralization on day 21 compared with the control groups. Furthermore, lactate was able to inhibit RANKL-induced osteoclast formation *in vitro*.

**Conclusion:** Lactate down-regulated the production of pro-inflammatory factors in the LPS-stimulated macrophages. In addition, lactate promoted osteogenic differentiation of both pre-osteoblasts and BMSCs, and inhibited osteoclastogenesis *in vitro*. Subsequently, we will focus on developing innovative bioengineering technologies to achieve more efficient delivery for potential bone tissue engineering applications.

Supported by: NIH R01 DE029159

## 29. *MiR-200c* Promotes Osteogenesis Synergistically with *miR-218*



**Chawin Upara**<sup>1</sup>, M. Zhu<sup>1</sup>, F. Shao<sup>1</sup>, H. Cao<sup>1</sup>, L. Hong<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** We have previously demonstrated that *miR-200c* can promote osteogenesis and regenerate bone in a critical-sized rat calvaria defect model. However, its

potency in regenerating mandibular bone is limited due to less vascularization and stem cell source. *MiR-218* has been reported to promote osteogenesis and inhibit osteoclastogenesis. In this study, we aim to characterize whether there are synergistic effects among *miR-200c* and *miR-218* on osteogenesis in order to enhance bone regeneration in the restoration of mandibular defects.

**Methods:** Human bone marrow stem cells (hBMSCs) were co-transfected with plasmid encoding *miR-200c* and *miR-218* using biocompatible calcium carbonate (CaCO<sub>3</sub>)-based nanoparticles and subsequently cultured in osteogenic differentiation medium. Transfection efficiencies of microRNAs and osteogenic biomarkers were quantified using RT-qPCR. Alkaline phosphatase and alizarin red S staining were also used to determine osteogenic differentiation and mineralization of hBMSCs after different treatments.

**Results:** CaCO<sub>3</sub>-based nanoparticles effectively transfect both *miR-200c* and *miR-218* into hBMSCs *in vitro* and enhance osteogenic differentiation biomarkers. Interestingly, co-transfection of both *miR-200c* and *miR-218* significantly increased *miR-200c* expression level compared to transfection with *miR-200c* alone. Additionally, co-transfected cells were more heavily stained on both the alkaline phosphatase and alizarin red S stain.

**Conclusion:** Co-transfection of *miR-200c* and *miR-218* concurrently promotes *miR-200c* expression and osteogenic differentiation of hBMSCs *in vitro* compared to overexpression of *miR-200c* alone.

Supported by: NIDCR R56 DE026433

### 30. Engineering Alpha-Ketoglutarate for Bone Regeneration



**Zhuozhi Wang**<sup>1</sup>, L. Zhong<sup>1</sup>, H. Sun<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** Alpha-ketoglutarate (AKG), a key Tricarboxylic acid cycle component, has garnered attention for its antiaging properties in various organisms. Our recent study has shown that Dimethyl alpha-ketoglutarate (DMAKG), a cell-permeable derivative of AKG, significantly promotes osteogenic differentiation and bone regeneration in aged mice. However, its cytotoxicity and rapid hydrolysis limit its application. To address these challenges, a new system which can sustainably deliver AKG directly into cells is to be developed.

**Methods:** Specifically, we designed and synthesized a novel polyester incorporating an AKG moiety (PAKG) to fabricate nanoparticles (NPs). Preosteoblast MC3T3-E1 cells and mouse bone marrow mesenchymal stem cells (mBMSCs) were cultured with NPs to test the osteoblastic/osteogenic differentiation and the subsequent mineralization. Furthermore, we developed a macro-porous microparticle (MPs) with PLGA and PAKG as scaffold for cell growth/differentiation and delivery. MC3T3-E1 and mBMSCs were seeded on the porous MPs. ALP activity and mineralization were also evaluated.

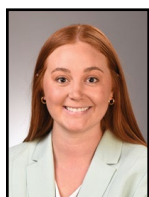
**Results:** Excitingly, these biodegradable PAKG NPs were highly phagocytosable for nonphagocytic cells (e.g., pre-osteoblasts MC3T3-E1 and primary bone marrow MSCs) while common poly (L-lactic acid) and poly (lactic-co-glycolic acid) MPs (PLLA & PLGA) with similar size fail to do so. *In vitro* studies demonstrated that PAKG NPs significantly promoted osteoblastic/osteogenic differentiation and subsequent mineralization in different cell types. The *in vitro* data also suggested that the chemical components, hydrophilicity, and the size of the particles significantly affected the cytotoxicity and pro-osteogenic activity. The *in vivo* studies showed that PAKG-based NPs significantly promote the cranial bone regeneration of mice. Moreover, the osteoblastic/osteogenic differentiation and subsequent mineralization in different cell types were significantly improved by the PLGA-PAKG porous MPs compared to PLGA porous MPs group.

**Conclusion:** In summary, the novel PAKG and the porous PLGA-PAKG MPs scaffold showed capabilities in osteogenic differentiation and bone regeneration and held great potential for broad regenerative medicine.

Supported by: NIDCR R01 DE029159

## Posters

### 31. Diet, Stress, and Sociodemographic Factors Impact on Pediatric Oral Health



**Kaitlynn Harvey**<sup>1</sup>, H. Steinkamp<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** Adverse Childhood Experiences (ACEs) are potentially traumatic events that can disrupt a child's neurodevelopment and trigger toxic stress. Prolonged activation of the stress-response system has been associated with various physical, emotional, and behavioral health challenges across the lifespan. ACEs encompass experiences such as abuse, neglect, household challenges, food insecurity, and other forms of instability. Research indicates that parents experiencing financial hardship are more prone to stress, depression, and family conflict—factors that significantly heighten the risk of ACE exposure. There is strong evidence linking low socioeconomic status to ACEs and subsequent long-term health, educational, and social outcomes for children.

**Methods:** This study seeks to examine the association between diet, stress, and sociodemographic factors by investigating a cohort of children aged 2-6 undergoing general anesthesia for routine elective surgical procedures at the University of Iowa Stead Family Children's Hospital.

Legal guardians of eligible children will complete a questionnaire addressing health, diet, oral hygiene practices, sociodemographic variables, and psychological distress.

**Conclusion:** This study aims to deepen our understanding of how diet, stress, and sociodemographic factors influence the oral health of young children undergoing general anesthesia for minor surgical procedures. By examining children from diverse backgrounds, the research will assess how factors such as socioeconomic status, parental stress, and dietary habits contribute to oral health outcomes. The findings are expected to highlight the critical role of early-life experiences and environmental influences in shaping oral health, underscoring the importance of addressing these factors in pediatric care. Ultimately, the study may inform strategies for improving oral health in at-risk populations, promoting healthier outcomes through targeted interventions and support for families facing socioeconomic or psychological challenges. Data collection, survey administration, and sample processing are currently ongoing.

### 32. Efficacy of Triazolam as a Sedative for Pediatric Dental Treatment



**Uzair Ahmad**<sup>1</sup>, *H. Steinkamp*<sup>1</sup>, *N. Odowa*<sup>1</sup>

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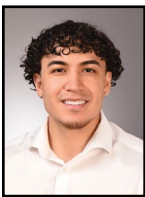
**Objective:** Pediatric dentists often employ oral sedation as a method of advanced pharmacological behavior management to safely treat uncooperative children with extensive needs. Two commonly employed sedatives are midazolam and diazepam. These benzodiazepines are popular due to their wide safety margin, rapid onset, reversibility and potential for anterograde amnesia. Triazolam is a benzodiazepine classified by the FDA as a hypnotic but has off label use for minimal/moderate sedation. It has a longer onset and half-life than midazolam, but shorter half-life than diazepam. This could make it a preferable drug for children. However, there are not many studies which investigate triazolam as a sedative for this population. The purpose of this study is to investigate the efficacy of triazolam as an oral sedative agent for uncooperative pediatric dental patients who require extensive dental treatment at the University of Iowa College of Dentistry Department of Pediatric Dentistry.

**Methods:** Subjects in this study are patients who had already been planned to undergo oral sedation with either triazolam or diazepam. In order to compare the effectiveness of the medications, researchers will utilize the Ramsey sedation scale to describe subjects behavior and sedation level throughout the appointment. We will also employ a 22-36 hour post-op questionnaire for the parents of the subjects

**Results:** We are in the early phase of data collection.

**Conclusion:** Results of this study could be used to help clinicians make evidence based decisions when selecting a drug regimen for pediatric oral sedation.

### 33. Lesion Sterilization and Tissue Repair Use by Pediatric Dental Providers



**Ted Rico**<sup>1</sup>, *M.K. Geneser*<sup>1</sup>, *O. Rysavy*<sup>1</sup>, *M. Matkovich*<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** The aim of this study was to assess the frequency of LSTR use among pediatric dentists, explore barriers to its adoption, and identify factors influencing its utilization.

**Methods:** A survey consisting of 19 questions was distributed via email to 6,636 members of the AAPD. The survey gathered demographic data, evaluated the current use of LSTR, and examined perceived barriers. Bivariate analyses using chi-squared and Fisher's exact tests examined associations between provider characteristics and LSTR usage.

**Results:** A total of 314 pediatric dentists completed the survey, with 26% reporting LSTR use. Bivariate analysis revealed that workplace setting was significantly associated with LSTR adoption ( $p = 0.010$ ); 50% of academic institution practitioners used LSTR compared to 26% in private practice. LSTR was primarily used for children aged 5–6 years (77%) and most commonly on primary second molars (54%). Clindamycin,

metronidazole, and ciprofloxacin was the most frequently used medicament (45%), with 57% using LSTR less than once per month. Among non-users (74%), the main barriers to LSTR adoption were lack of training (69%) and a preference for traditional treatments like pulpectomy or extraction (32%). Majority of the respondents who reported never using LSTR expressed interest in continuing education courses (85%).

**Conclusion:** LSTR remains underutilized among pediatric dentists due to lack of training and/or a preference for traditional treatments. The variability in usage highlight the need for targeted training programs, and broader dissemination of evidence-based guidelines to address knowledge gaps and improve confidence in its clinical use for necrotic primary molars.

### 34. Rare Multifocal Oral EBV-Positive Mucocutaneous Ulcer: Case Series



**Kittiphoj Tikhanarak**<sup>1</sup>, *J. Saepoo*<sup>1</sup>, *E. Lanzel*<sup>1</sup>, *S. Timmons*<sup>1</sup>, *J.W. Hellstein*<sup>1</sup>, *N. Handoo*<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** Epstein-Barr virus-positive mucocutaneous ulcer (EBV-MCU) is a rare, self-limiting lymphoproliferative disorder often seen in immunosuppressed individuals, including the elderly. It can involve the oral mucosa and mimic malignant lesions clinically and histologically. While solitary lesions are most commonly reported, multifocal presentations are exceedingly rare, with only two previously documented oral cases. This series highlights one multifocal and three solitary oral EBV-MCU cases, focusing on clinical features, diagnostic challenges, and management strategies.

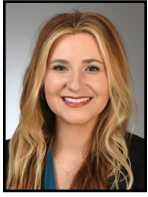
**Methods:** We conducted a retrospective analysis of cases submitted to the Oral Pathology Clinic and Laboratory at the University of Iowa, diagnosed as EBV-positive mucocutaneous ulcer.

**Results:** An 80-year-old patient presented with multiple oral ulcers. Histopathological analysis revealed ulceration with granulation tissue, atypical lymphoid cells positive for CD20 (IHC) and PAX5 (IHC). Epstein-Barr virus was confirmed by EBV-encoded RNA (EBER) in situ hybridization. One month later, the patient was diagnosed with diffuse large B-cell lymphoma (DLBCL), underscoring the importance of immune status evaluation. Three additional cases were identified presenting as solitary oral ulcers at different sites, with no immunosuppression reported, and sharing similar histopathological findings. In these 3 patients, medical work-up did not reveal any underlying immunosuppressive diseases and the patients experienced resolution of EBV-MCU with supportive care.

**Conclusion:** This case series underscores the rarity of multifocal EBV-MCU and its potential association with underlying conditions. Awareness among clinicians and pathologists is essential to recognize this benign lesion, particularly in the elderly, that can mimic malignancy. While EBV-MCU typically resolves spontaneously with a favorable prognosis, thorough evaluation for potential immune dysregulation or systemic disease is critical, particularly in multifocal lesions.



### 35. Retention Comparison of Dental Sealants Using Cured Versus Uncured Adhesive



**Michaela Mathews**<sup>1</sup>, C.M.P. Vidal<sup>1</sup>,  
H. Steinkamp<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** This study aims to evaluate the retention differences of dental sealants when using uncured versus cured dental adhesive.

This is an important consideration in pediatric patients where additional cure time may increase the likelihood of moisture contamination. The clinical success and necessity of dental sealants are well-documented in the literature, with systematic reviews and meta-analyses highlighting their efficacy in preventing occlusal cavities in children and adolescents. These findings underscore the importance of optimizing sealant application techniques to enhance their longevity and effectiveness in occlusal cavity prevention.

**Methods:** Twelve previously extracted molar teeth were sectioned into two portions with an exposed, smooth, enamel, occlusal surface. Twenty-four halves were each divided into two separate corresponding groups – uncured bond and cured bond for control. The occlusal surfaces were smoothed for a fresh layer of enamel and prepared for sealant application. Both groups underwent identical surface preparation with a 37% phosphoric acid etchant applied for 30 seconds, followed by thorough rinsing, and drying. In the cured adhesive group, Optibond adhesive was applied and cured for 20 seconds. In the uncured adhesive group, Optibond adhesive was applied without curing. For both groups, Clinpro (3M ESPE) sealant was placed on top of the adhesive and cured for 20 seconds. Shear bond strength of the samples was tested to assess retention in megapascals.

**Results:** Statistical analysis was conducted to evaluate the retention differences of dental sealants when using cured versus uncured adhesive. No significant difference was observed between the two groups ( $p=0.053$ ). Normality was confirmed for both groups with the Shapiro-Wilk test. A one-tailed two-sample t-test was performed assessing if retention for uncured adhesive is greater than cured adhesive. Analysis results: T-Value= -1.68, DF= 21, P-Value= 0.053

**Conclusion:** This study seeks to provide insight into whether curing dental adhesive prior to sealant application improves retention or if uncured adhesive offers comparable performance, potentially reducing the risk of moisture contamination during application in pediatric settings. While the results suggest a trend toward greater retention for uncured adhesive compared to cured adhesive, the evidence is not strong enough to conclusively reject the null hypothesis ( $p$ -value 0.053). Further investigation with larger sample sizes is recommended to confirm these findings and provide greater statistical power.

### 36. Americans Have Low Dental Insurance Literacy



**Benjamin Pierson**<sup>1</sup>, W. Shi<sup>1</sup>,  
M.R. McQuistan<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** Dental insurance terminology and coverage can be difficult to understand. The aim of this study was to investigate dental insurance literacy in the United States.

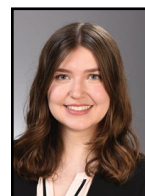
**Methods:** A 48 item survey was developed and received IRB approval. The survey assessed participants' knowledge of dental insurance terminology, ability to calculate out-of-pocket costs for treatment, and feelings about choosing and using dental insurance (scale: 1=not very likely/confident to 7=very likely/confident). The Qualtrics survey was posted in August 2024 on Amazon Mechanical Turk. Respondents received \$3 for participating. The total number of correct answers were calculated for knowledge (maximum=10) and out-of-pocket costs (maximum=5), while mean scores were calculated for choosing and using dental insurance. Descriptive statistics were calculated. Bivariate and multivariate regression analyses were conducted to assess associations with knowledge and out-of-pocket costs.

**Results:** N=166. The majority of respondents were male (74%) and white (95%), while 37% indicated they were Latino. Ages ranged from 21-51 years. The mean knowledge score was 5.46/10, and the mean calculating costs for treatment score was 1.74/5. The mean scores for choosing and using a dental insurance plan were 5.55/7 and 5.56/7, which were similar to confidence in choosing (5.61/7) and using a dental insurance plan (5.58/7). Respondents who reported low confidence filling out medical forms, a longer amount of time since their last dental visit, or an income <\$50,000 were the most likely to have low dental insurance knowledge. Respondents who have received treatment at places other than a private practice dentist's office, lived in the United States for less than 5 years, and had private health insurance only were the most likely to incorrectly calculate out-of-pocket costs.

**Conclusion:** Although respondents felt confident choosing and using dental insurance, the mean knowledge and out-of-pocket costs calculation scores indicate that respondents had low dental insurance literacy.

Supported by: University of Iowa College of Dentistry Student Research Program; M.W. Finkelstein Teaching Professorship

### 37. Anticariogenic Properties of Probiotic Candidates



**Veronica C. McClennen**<sup>1</sup>, J.A. Banas<sup>1</sup>,  
M. Zhu<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** Dental caries, caused by a dysbiosis in the plaque microbiome, are highly prevalent throughout the world. Probiotics are an option to restore a healthy microbial balance to the mouth. We hypothesize that candidates capable of strong adhesion to salivary pellicle and/or synthesis of hydrogen peroxide will be best capable of preventing *Streptococcus*

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*mutans*-based caries. The objective of this study was to identify probiotic candidates capable of strong adhesion to salivary pellicle and/or hydrogen peroxide production and test them for suppression of *S. mutans* cariogenicity.

**Methods:** Probiotic candidates obtained as clinical isolates from caries-free children and shown to metabolize arginine to base were further analyzed for properties desirable in a probiotic. Emphasis was placed on ability to adhere to salivary pellicle, which was tested in triplicate in-vitro using individual and pooled human saliva samples in an assay that quantified biofilm biomass after 48-hour growth. The amount of hydrogen peroxide production was assayed and compared to that produced by *Streptococcus sanguinis*, a known *S. mutans* antagonist. The ability to reduce the cariogenicity of *S. mutans* was tested in duplicate in an ex-vivo model using mouse jaws. Confocal microscopy was used for analysis of biofilm architecture.

**Results:** Strong biofilm formation was observed with pooled saliva samples, but displayed considerable heterogeneity – up to 12-fold – with individual samples. Overall, pellicle binding proficiency was more strongly linked to inhibition of *S. mutans* cariogenicity than was the quantity of hydrogen peroxide produced. Reductions in cariogenicity were associated with two-fold or greater reductions in overall biofilm thickness.

**Conclusion:** Probiotic adhesion to pellicle was linked to a reduction in *S. mutans* biofilm thickness and a reduction in *S. mutans* cariogenicity. When considering probiotic selection, strains with broadly strong pellicle adhesion would be most ideal, although individualized selection based on host receptiveness would also optimize efficacy.

Supported by: NIDCR R56 DE026716; University of Iowa College of Dentistry Student Research Program

### 38. Dental Treatment Among Older Adults with Mental Health Disorders



**Katie McAllister**<sup>1</sup>, F. Qian<sup>1</sup>, L. Marchini<sup>1</sup>, J. Tate<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** To describe the most common dental treatment among older adults with mental health disorders, and what variables are associated with the frequency of annual preventive dental visits.

**Methods:** Data was extracted from electronic health records of University of Iowa College of Dentistry for all individuals aged 65 years or older that self-reported a mental health condition, between 2008 and 2024. Descriptive statistics were used to summarize the demographic and clinical characteristics of the study participants, while significant predictors of frequency of annual preventive dental visits (<2 average-visits per-year vs. ≥2) were identified through multivariable logistic regression analysis (alpha=0.05).

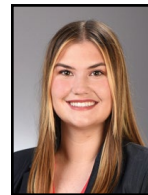
**Results:** A total of 3,846 subjects met the inclusion criteria. From this total, 1,853 (48.2%) received preventative dental services and 1,294 (33.6%) had at least one annual preventive dental visit. Of this group, 64.2% were females, average age was 76.0±9.1 years, 54.3% were self-paying, 62.1% resided within a 30 miles distance from the clinic, and 70.9% were treated by pre-doctoral students. Nearly 43% of patients

were obese and took 7.8±7.2 daily medications. The average number of teeth was 25.0±7.3. Additionally, 83.7% of the patients had heart disease, 54.9% xerostomia, and 5.3% history of drug addiction. Approximately 40% used tobacco, 45.7% had diabetes, and 30.7% struggled with alcohol addiction. For each year of age, the odds of attending two or more annual preventive dental services increased by 4% (OR=1.04, 95% CI 1.01-1.08; p=0.004). Patients who were treated by faculty members had higher odds of attending two or more annual preventive dental visits compared to those treated by predoctoral students (OR=3.77, 95% CI 2.24-6.36; p<0.001).

**Conclusion:** In this sample of older adults with mental health conditions, about one-third had at least one annual preventive dental visit during the observation period. Receiving care from faculty members significantly increased the odds of attending annual preventive dental visits.

Supported by: University of Iowa College of Dentistry Student Research Program

### 39. Effect of Ibuprofen and Acetaminophen on Baseline Pulpal Sensibility



**Madison Thatcher**<sup>1</sup>, C. Hogden<sup>1</sup>, F. Qian<sup>1</sup>, R. Bowers<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** Pain is frequently reported during dental procedures despite the use of local anesthesia. Clinical research has demonstrated that over-the-counter pain medication, such as ibuprofen and acetaminophen, increases pain thresholds and/or tolerance in cases with severe inflammation. However, there is little understanding regarding their impact on non-inflammatory states. The objective of our study was to assess the significance of oral ibuprofen and acetaminophen on pulpal pain thresholds in routine care scenarios.

**Methods:** A double-blinded randomized cross-over clinical investigation assessed the effect of a combination of oral ibuprofen and acetaminophen (IBU-APAP) compared to a placebo control on electric pulp test (EPT) response thresholds. Three non-carious/restored teeth/per patient (central incisor, maxillary premolar, and mandibular premolar) were randomly selected. Periodontal probing was completed with bleeding on probing (BOP) recorded. EPT threshold values for each tooth were measured at five time points following ingestion of assigned capsules (0, 10, 20, 30, and 40 minutes). Statistical analysis was conducted using a two-sample t-test, paired-sample t-test, Wilcoxon rank-sum test, and Wilcoxon signed-rank test (α=0.05).

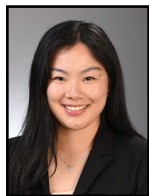
**Results:** Forty participants completed the study (75% female, mean age 26.2 ± 5.3 years, BOP 22.5%). No significant differences were found between IBU-APAP and placebo treatments across time points for each tooth type except in one case. At 40 minutes, mean/median EPT thresholds for maxillary premolar teeth under the IBU-APAP were significantly higher than the placebo (42.85±14.15\40.00 vs. 37.83±12.31\35.50; p=0.035).

**Conclusion:** Our study found a small potential pain threshold impact of IBU-APAP compared to placebo. The large standard deviations in the data reflect significant variation in pain thresholds amongst the teeth assessed. The limited

distribution of gender and BOP reduced the statistical power for analyzing these characteristics. Further objective assessments on the analgesic effects of these medications in non-inflammatory and routine dental treatment scenarios are warranted to better determine their clinical relevance.

Supported by: University of Iowa College of Dentistry Student Research Program

#### 40. Evaluating the Adhesion of *Streptococcus mutans* to Additive Manufactured Zirconia



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<sup>1</sup>University of Iowa, Iowa City, IA; <sup>114</sup>University of Texas Health Science Center at Houston School of Dentistry, Houston, TX

**Objective:** The use of computer-aided design and computer-aided manufacturing technology has enabled the precise fabrication of restorations. Zirconia has emerged as an important material used to manufacture all-ceramic restorations by traditional milling. However, traditional milling presents challenges such as material waste, limited surface detail, and persistent high costs associated with milling bur turnover. Additive manufacturing is a promising alternative that could be the future on how most restorative materials can be developed and 3-D printed using zirconia. The advantage of this technology lies in its ability to fabricate materials with different porosities, which has implications for material shrinkage, and promoting stronger bonding. A recent study evaluated the roughness and the bond strength of additively manufactured (AM) zirconia. However, there are no studies that evaluated the microbial adhesion properties of AM. The objective of this study is to compare the microbial adhesion of streptococcus mutans between AM and milled zirconia (MZG) fabricated discs.

**Methods:** A 12 x 5 mm disc digital STL file was generated to fabricate zirconia discs by additive manufactured technology and traditional milling. AM discs were made with a porosity of 50 µm (AM-50G). The roughness (Ra) of all discs was measured using a profilometer. Subsequently, we intend to measure the adherence of *S. mutans* to AM and MZG fabricated discs. We propose to expose 9 discs for each fabrication in the presence of *S. mutans* and 3 AM and MZG discs as negative controls to evaluate the sterility.

**Results:** The average Ra was 1405.0 µm for AM-50G and 1243.0 µm for MZG, respectively. Based on the Ra values, we anticipate a significantly higher number of bacteria will adhere to the AM-50G discs compared to the MZG discs.

**Conclusion:** This study will provide insights on how bacteria attach to additive manufactured discs based on the surface properties.

Supported by: University of Iowa College of Dentistry Student Research Program

#### 41. Impact of Porosity on Dimensional Accuracy of 3D-Printed Yttria-Stabilized Tetragonal



**Shubhi Sharma**<sup>1</sup>, A. Zandinejad<sup>227</sup>, S.H. Cho<sup>1</sup>, F. Floriani<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA; <sup>227</sup>University of Rochester, Rochester, NY

**Objective:** The integration of digital dental materials, particularly 3D-printed zirconia, represents a transformative advancement in modern dentistry. Despite the wide acceptance and use of milled zirconia, its transition to 3D printing aims to overcome some inherent limitations, promising a bright future for dental restorations. This study aims to analyze the mechanical properties of a newly developed 3D printable zirconia.

**Methods:** Digital STL file was generated using a software, incorporating dimensions of 12 diameter x 5 mm. For the experimental phase, the test disks (n=20) consisted of two distinct groups (n=10). Groups were separated by no designed porosity (Control) and design pores of 50x50 microns 200 µm apart (G1:50). The designs were exported as a standard tessellation language (STL) file and used to additively manufacture (AM) the disks with different surface textures using an SLA 3D printer and a zirconia material (3DMix ZrO2 paste, 3DCeram Co). The diameter and thickness of all AM specimens were measured with digital calipers. The profilometer was used to measure the average roughness (Ra) of each specimen. Each sample's microstructural surface characterization was evaluated using a Scanning Electron Microscope (SEM). Mean, standard deviation was calculated for each group regarding the volumetric changes (diameter and thickness). Data presented a normal distribution (Shapiro-Wilk test, p>0.05). The groups were compared using an independent-sample t-test.

**Results:** The significance level adopted was p<0.05 for all tests. There was a significant difference in diameter (t= -4.546; p<0.001) with higher mean values for the "rough" group, and no statistical difference for thickness (t= 0.470; p=0.644).

**Conclusion:** While additive manufacturing presents a viable alternative to traditional subtractive methods for fabricating zirconia crowns, careful consideration must be given to the design parameters and the control of the manufacturing process to ensure dimensional accuracy and material integrity while customizing them for better clinical outcomes.

Supported by: University of Iowa College of Dentistry Student Research Program

#### 42. Weight Loss Strategies and Their Associations with Oral Health



**Katy Volz**<sup>1</sup>, D.J. Caplan<sup>1</sup>, J. Desai<sup>1</sup>, F. Qian<sup>1</sup>, T.A. Marshall<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** In addition to gastric bypass surgery (GBS), weight loss medications (WLM) have been introduced and marketed to consumers, yet their implications for oral health have not been well-described. We conducted a feasibility study to explore associations between GBS and/or WLM and

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oral health. Herein, we report associations between GBS and/or WLM and caries outcomes (Decayed (D), Missing (M), Filled (F), Teeth (T)).

**Methods:** Study subjects included Iowa's College of Dentistry patients aged 18+ years with a comprehensive exam between 2017 and 2024. Cases were defined by a self-reported history of GBS or use of at least one WLM; controls reported no history of GBS or WLM use as of their exam dates. Each case was matched with two age- and sex controls. Axium records were screened to identify cases (n= 376) and demographic, health, and dental variables were collected. Five percent (18 matched pairs) of case records were randomly selected for chart note screening. Data extracted from Axium records were manually screened for accuracy, Axium searches were refined to improve accuracy, and the process was repeated until accurate Axium extraction was achieved electronically. One control was found to be matched to two unique case subjects, so one of those control observations was not analyzed.

**Results:** Among our 53 subjects, 56% were female and the mean age was 53 years. Of our 18 cases, 1 reported a history of GBS and 17 of WLM use. BMI of cases (n=17 pairs) trended higher than controls (p=0.089). However, the DMFT of cases (n=18) was similar to controls (p=0.699) as were the number of D teeth (p=0.728), F teeth (p=0.435), and M teeth (p=0.551).

**Conclusion:** DMFT values were not associated with GBS or WLM use in our feasibility sample. Additional investigation of associations between weight loss therapies and oral health outcomes is warranted.

Supported by: University of Iowa College of Dentistry Student Research Program

### 43. Weight Loss Therapies and Oral Health in Adults: NHANES Analysis



**Keshav Wagle**<sup>1</sup>, C. Pendleton<sup>1</sup>, S.C. McKernan<sup>50</sup>, S.M. Ganesan<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA; <sup>50</sup>University of Minnesota, Minneapolis, MN

**Objective:** Obesity affects 41.9% of U.S. adults, and numerous studies have linked obesity to poorer oral health outcomes.

However, the effect of weight loss therapies on oral health has not been thoroughly explored. The National Health and Nutrition Examination Survey (NHANES) offers valuable data to investigate associations between obesity, weight loss efforts, and self-reported oral health in a large, diverse population. This study aims to examine how weight loss behaviors influence self-reported oral health perceptions and dental utilization among U.S. adults.

**Methods:** Data from NHANES 2017–March 2020 was analyzed. Adults were categorized into four BMI groups: healthy weight, overweight, obese, and severely obese. Information on intentional weight loss behaviors (e.g., dieting, exercising) and self-reported success in weight loss was collected. Oral health outcomes, including self-reported oral health and periodontal disease, were assessed. Statistical analysis was conducted using Wilcoxon rank sum tests, Kruskal-Wallis tests, Chi-squared tests, and regression analysis to evaluate associations between BMI, weight loss strategies, and oral health outcomes.

**Results:** Obese and severely obese participants were significantly more likely to report fair or poor oral health compared to those with a healthy BMI (p < 0.05). Participants who attempted weight loss, particularly those who used weight loss programs, increased fruit/vegetable intake, or exercised, reported significantly better oral health perceptions. Weight loss therapies that involved lifestyle changes demonstrated a stronger association with improved oral health perceptions than other methods.

**Conclusion:** This study underscores the connection between weight status and oral health perceptions. Importantly, intentional weight loss strategies, particularly those focused on improving systemic health, were linked to better self-reported oral health outcomes. These findings highlight the need for integrated healthcare approaches that address both obesity and oral health to enhance overall well-being.

Supported by: University of Iowa College of Dentistry Student Research Program

### 44. Weight Stigma & Bias Amongst Dental Students



**Elise A. Amerongen**<sup>1</sup>, T.A. Marshall<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** Reinforced by a societal emphasis on thinness, individuals with obesity face stereotypes, biases, and prejudices (SBP). These SBP are also observed among healthcare providers. Our objective was to describe obesity SBP among dental students. We hypothesize that obesity SBP among students is similar to SBP observed among other healthcare employees.

**Methods:** D1-D4 dental students (n=333) attending the University of Iowa in the Fall 2024 were invited to participate. A survey designed to query demographics and obesity SBP was administered using QualtricsÆ survey platform. Obesity SBP was assessed using the validated Fat Phobia Scale and Antifat Attitudes Questionnaire.

**Results:** Responding students (n=94) were 93.6% under age 30, 68.1% female, and 81.9% white. Most respondents disagreed with the statement: "I tend to think that people who are obese are a little untrustworthy" (86.5%). Most respondents agreed with the statement: "People who weigh too much could lose at least some part of their weight through a little exercise" (62.9%). Students were evenly distributed on the statement: "One of the worst things that could happen to me would be if I gained 25 pounds" (disagree= 30.3%; neutral= 23.8%; agree= 46.1%). A majority of students favored negative descriptions of obese individuals; for example, 'lazy' (n=47) over 'industrious' (n=8) and having 'poor self-control' (n=54) over 'good self-control' (n=10).

**Conclusion:** Many dental students hold some level of obesity SBP, which is consistent with other health care providers. While the majority of students disagreed with anti-obesity trust and honesty statements, there was significant agreement with other anti-obesity statements and negative descriptors. Equally concerning was the level of agreement with a fear of becoming obese.

Supported by: University of Iowa College of Dentistry Student Research Program

## 45. Associations Between Water Insecurity and Beverage Intakes in Pediatric Patients



**Maya G. Olson**<sup>1</sup>, T.A. Marshall<sup>1</sup>,  
H. Steinkamp<sup>1</sup>, F. Qian<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** Water insecurity can be defined as the inability to reliably access and benefit from safe water for all household uses. Families experiencing water insecurity may be inclined

to consume other beverages including sugar-sweetened beverages (SSB) instead of water, which has implications for caries risk. This project is designed to explore associations between water security perceptions and beverage intakes amongst a pediatric population.

**Methods:** Parents of children aged 2-8 presenting for a new or recall appointment were recruited from the University of Iowa College of Dentistry's Pediatric Clinic for an ongoing study. Parents completed an anonymous Qualtrics survey querying family demographics, parental perceptions of water security, and their children's beverage intakes (Child's Beverage Intake Questionnaire). Statistical analysis included both descriptive and bivariate analyses (alpha=0.05).

**Results:** Children (n=87) had a mean age of 5.4±1.4 years, 54% were White, 40% reported food insecurity, and nearly 34% of parents had education attainment of 8th grade or below. Approximately 69% of participants believed their home tap water was safe to drink, 57% felt bottled water was safer than tap water, and 60% said their home tap water tasted good. White participants were more likely than non-white participants to agree that their home tap water was safe to drink (p=0.043). Hispanic participants were more likely than non-Hispanic participants to give their children drink bottled water (p=0.032). Children using public water sources consumed less water than those with private or community sources (p=0.031). Those who primarily drank bottled water at home consumed significantly more SSBs (p=0.028) and 100% juice (p=0.002) than children who drank tap water.

**Conclusion:** Children of participants who used bottled water at home consumed significantly more SSBs and 100% juice; behaviors associated with water insecurity that might increase the risk of caries.

Supported by: M.W. Finkelstein Teaching Professorship; University of Iowa College of Dentistry Student Research Program

## 46. Awareness of Non-Carious Cervical Lesions Among Dental Students and Faculty



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**Objective:** Non-Carious Cervical Lesions (NCCLs) characterize a condition where dental hard tissue is progressively lost at the cemento-enamel junction and are multi-

factorial in nature. The prevalence of NCCLs is rising in patients, leading to increased susceptibility to pain, functional limitations, and poor esthetics. Despite this, few dentists

feel prepared to diagnose NCCLs and provide intervention. Early identification allows for appropriate treatment that can preserve tooth integrity and improve patient outcomes in dental health. The objective of this study is to examine the general knowledge of NCCLs among dental students, residents, and faculty within the University of Iowa College of Dentistry.

**Methods:** Students (n= 146), residents (n= 7), and faculty (n= 36) at the University of Iowa College of Dentistry were recruited to take part in a survey about Non-Carious Cervical Lesions. The survey was administered using Qualtrics software and utilized 10 questions to assess the knowledge participants held about the etiology of NCCLs, existing preventative methods, and confidence participants felt in clinical diagnosis of NCCLs. The survey data was analyzed using Fisher's Exact tests and the R software.

**Results:** 75% of respondents at the College of Dentistry had heard of NCCLs, yet 43% reported being uncertain how they develop. Only 29% of participants felt prepared to diagnose NCCLs in initial and advanced stages. Statistically significant differences were found between student, resident, and faculty perceptions of etiology (p < 0.002), clinical experience with NCCLs (p < 0.001), and preparedness to diagnose (p < 0.001).

**Conclusion:** Conflicting results of knowledge about the etiology and clinical diagnosis of NCCLs could be observed in this population involving students, residents, and faculty. The study indicates that dental students, residents, and faculty may benefit from more comprehensive education on this topic.

Supported by: University of Iowa College of Dentistry Student Research Program

## 47. Classifications of Reported Incidents at a Dental College



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**Objective:** To characterize potential medical and procedural incidents reported within the University of Iowa College of Dentistry (UICOD).

**Methods:** A review was completed of all incident reports from January 1, 2022 through June 30, 2024. Reports included incidents for patients and nonpatients. Information gathered included age, gender, day of the week, time, type of event, clinic, who responded to the incident, and whether an ambulance was called. Additional information was gathered for patient incidents including number of medications, chronic illnesses, when the incident occurred in relation to the procedure, and whether patient was a comprehensive care patient or an emergency patient. Descriptive statistics were used to quantify the data.

**Results:** A total of 74 reports were reviewed with an average age of 55. Females comprised two-thirds of the incidents. Most incidents with patients happened mid-procedure (65%) with the remaining events split evenly between pre- and post-treatment. The most common medical incidents were syncope (n=11) and falls (n=10), followed by seizures (n=7) and hypertensive episodes (n=6). Forty-seven percent of patients reported having 4 or more chronic illnesses. Forty-six percent of patients were medicated for 3 or more systems.

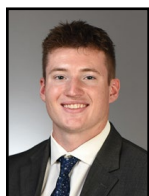
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The most common medications were for cardiovascular problems followed by medications for behavioral, developmental, or mental health. The most common chronic illnesses were from cardiovascular and muscle, bone, and connective tissues groups.

**Conclusion:** Results are used to help identify risks in both patients and non-patients, prevent similar incidents, and increase the culture of safety within the University of Iowa College of Dentistry.

Supported by: University of Iowa College of Dentistry Student Research Program

#### 48. Compositional Changes of Teeth with Wedge-Shaped Noncarious Cervical Lesions



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**Objective:** To address the knowledge gap regarding dental tissue integrity in teeth with wedge-shaped noncarious cervical lesions (NCCLs), this study evaluated differences in

organic composition and mineralization level between sound and NCCL-affected teeth.

**Methods:** Extracted teeth obtained from the biorepository at the University of Iowa College of Dentistry were examined to verify the presence or not of an NCCL. Selected teeth, consisting of five sound and five moderate NCCL (> 1mm deep lesion), were cut into halves (mesial and distal), embedded in epoxy, and analyzed for surface chemical characterization under RAMAN spectroscopy. Fifteen spectra were analyzed for each condition (sound and NCCL) and location (crown and root, dentin and enamel). Spectral analyses generated compositional ratios: mineral-to-matrix ratio (MMR), A vs. B type hydroxyapatite, proteoglycans, collagen cross-linking, and glycation. Compositional ratio averages were compared between sound and NCCL-affected samples and evaluated using Two-way Anova and Tukey's post-hoc ( $\alpha = 0.05$ ).

**Results:** NCCL-affected coronal and root dentin had a lower carbonate-to-hydroxyapatite ratio than sound ( $p=0.01$ ). Coronal dentin had a higher MMR ( $p=0.019$ ) compared to root dentin for both sound and NCCL-affected specimens. Regarding organic compositional differences, sound root dentin had a lower Amide1-to-CH2 ratio (collagen structure) compared to NCCL-affected root dentin ( $p=0.008$ ). NCCL-affected coronal and root dentin had lower proteoglycans-to-collagen ratios compared to sound specimens ( $p<0.001$ ). There were no significant differences between the sound and NCCL-affected samples for glycation or the mineralization ratios used for enamel ( $p>0.05$ ).

**Conclusion:** Multiple differences in the mineralization and organic composition between sound and NCCL-affected teeth were highlighted. NCCL-affected teeth have lower hydroxyapatite carbonation and lower levels of proteoglycans within the dentin extracellular matrix compared to sound teeth. Increased collagen structure ratio was observed in root dentin of NCCL specimens. Coronal dentin had higher MMR when compared to root dentin.

Supported by: University of Iowa College of Dentistry Student Research Program

#### 49. Custom Fabrication of Mini-Screw Assisted Rapid Palatal Expander (MARPE)



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**Objective:** Traditional rapid maxillary expansion (RME) devices lose efficacy after the pubertal growth spurt, typically around age 14. Mini-screw Assisted Rapid Palatal Expanders (MARPEs) offer a non-surgical alternative for older patients, but commercially available MARPEs may not accommodate variations in palatal anatomy, shape, or the location of vital structures. The University of Iowa Orthodontics Department developed a customizable MARPE fabrication technique, which optimizes temporary anchorage device (TAD) placement and jackscrew design. This approach allows for precise localization of TADs in areas of high bone density, while also accommodating patients with unique palatal morphology and avoiding critical anatomical structures.

**Methods:** The fabrication process begins with creating a cast model of the maxilla, which is used throughout the design phase. TAD locations are determined using the model, and implant analogues are positioned accordingly. Sectioned capillary tubes are then placed over the analogues, and a standard Hyrax jackscrew is laser-welded to these tubes. Finally, molar bands are soldered to the jackscrew on the cast. For appliance delivery, the molar bands are cemented in place, local anesthesia is administered near the TAD placement sites, and the TADs are inserted with bicortical penetration of the maxilla. Treatment success is indicated by the development of an interincisal diastema or sutural expansion on occlusal radiographs.

**Results:** Four cases involving patients aged 16-18 demonstrated successful midpalatal suture separation, a result typically unachievable with traditional RME devices in this age group.

**Conclusion:** The custom MARPE fabrication technique enables maxillary expansion in patients past the pubertal growth window, particularly those with unique palatal anatomies or vital structures that preclude the use of commercial MARPE devices. This approach expands the applicability of MARPEs, offering a tailored solution for complex cases.

Supported by: University of Iowa College of Dentistry Student Research Program

#### 50. Effects of Silver Diamine Fluoride Application Methodologies on Dentin Microhardness



**Jessica Grundmeyer**<sup>1</sup>, E. Araujo<sup>1</sup>, F. Qian<sup>1</sup>,  
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**Objective:** Silver diamine fluoride (SDF) is widely used in its off-label application in arresting caries. However, its effectiveness in restoring dentin microhardness remains unclear. This study aims to evaluate the impact of various SDF application methodologies on dentin surface microhardness



recovery, including application protocol time and the use of adjunctive materials.

**Methods:** Forty dentin samples (4x4x3 mm) were sectioned from human premolars. Artificial carious lesions (~100µm) were created under a demineralization-remineralization solution cycling for 14 days. The samples (n=4) were subjected to ten different SDF application protocols: negative control, 30-second SDF, 2-minute SDF, Acid etch (A/E) + 30-second SDF, A/E + 2-minute SDF, 30-second SDF + potassium iodide (KI), 2-minute SDF + KI, A/E + 30-second SDF + KI, and A/E + 2-minute SDF + KI. Surface microhardness was measured pre- and post-treatment using a Vickers microhardness tester. Data were analyzed using mixed model ANOVA and post-hoc pairwise comparisons (p<0.05).

**Results:** Statistically significant increases in microhardness from pre-treatment to post-treatment were observed in the groups treated with 30-second SDF (8.77±0.99 vs.11.73±0.82, change:2.96±0.27 Hv; p=0.002), 2-minute SDF (8.84±1.25 vs.12.82±0.89, change:3.99±0.83 Hv; p=0.017), and A/E followed by 30-second SDF (8.75±1.20 vs.15.26±0.37, change:6.51±0.97 Hv, p=0.007). No statistically significant changes in microhardness were found in the other groups.

**Conclusion:** While specific methods of SDF application resulted in statistically significant increases in dentin microhardness, the overall clinical significance of these findings is limited, as non-carious dentin typically measures >50 Hv. Given time and adjunctive treatments demonstrated a limited impact, taking additional measures or time when applying SDF does not appear to improve dentin microhardness restoration. However, there may be a more substantial benefit through biofilm inhibition, which could be potentiated by the precipitates formed after SDF application and is the subject of ongoing studies.

Supported by: University of Iowa College of Dentistry Student Research Program

## 51. Flange Design Impacts on Force Dislodgement of Maxillary Complete Dentures



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**Objective:** Flange border anatomy of complete dentures lacks objective evidence relating to the impact resulting from errors/decisions made by clinicians or laboratories in the preserving border-molded anatomy (i.e. thinning/polishing during processing). Our study quantitatively assesses dislodgement forces associated with different anatomical border flange thicknesses in maxillary complete dentures.

**Methods:** Edentulous patients treatment planned for complete maxillary denture fabrication are being screened in ongoing study. Baseline measurements included patient saliva quality, ridge form & mobility, and compressibility of posterior palate. Four 3D-printed base plates, each with same flange height but varying flange thicknesses: (A) all preserved, (B) thinned anterior segment, (C) thinned posterior segment, and (D) all segments thinned, were fabricated following border-molded master impressions. Pull attachments over the right

and left first premolar areas are used to attach a tension measuring device to measure the max force of dislodgement of each baseplate. Measurements were completed twice from each attachment of the baseplates, and the average of two measurements were used for the analysis. Descriptive statistics were completed on the initial data due to the current small sample size.

**Results:** In the ongoing study, six patients have completed the pull test (mean age=56.8±12.2 years, male 50%). Clinical examination results found 83% of patients had adequate saliva flow. Flat (33%) and U-shapes (67%) were noted, with ridge mobility observed in one patient. All patients had compressibility of their posterior palate. Comparisons of flange thickness showed minimal differences in the mean pull force values, with a high standard deviation noted across all groups (A=8.78±6.54N, 95% CI=1.91-15.65; B=10.02±7.01N, 95% CI=2.66-17.37; C=8.85±6.08N, 95% CI=2.47-15.22; D=9.47±7.14N, 95% CI=1.97-16.96).

**Conclusion:** Initial pull test findings show minimal differences between flange thicknesses and high variability of the assessed forced dislodgement. Further analyses using the complete data will be conducted.

Supported by: University of Iowa College of Dentistry Student Research Program

## 52. MMP-3/TIMP-4 Levels in Pulp Stem Cells Exposed to Bacterial By-Products



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**Objective:** This study aimed to investigate the matrix metalloproteases (MMP) and their tissue inhibitors (TIMP) balance in dental pulp in response to bacterial infection associate with caries. Specifically, the study focused on assessing the changes in protein levels of MMP-3 and TIMP-4 in dental pulp stem cells (DPSCs) stimulated by different bacterial by-products *in vitro*.

**Methods:** DPSCs were cultured in alpha-minimal essential medium (α-MEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution. Cells were exposed to bacterial by-products and filtrates for 24 hours (n=6). The bacterial stimuli included *Escherichia coli* lipopolysaccharide (LPS) at 10 µg/mL, *Bacillus subtilis* lipoteichoic acid (LTA) at 15 µg/mL, and filter-sterilized supernatants from *Streptococcus mutans* (SM) and *Lactobacillus rhamnosus* (LR) diluted to 20% in cell culture media. SM and LR filtrates were obtained by growing pure bacterial isolates on blood agar for 48 hours, followed by 24 hours in Dulbecco's modified Eagle medium (DMEM) containing 2% FBS and 1% MEM vitamins. MMP-3 and TIMP-4 protein levels were quantified using ELISA and multiplex bead-based assays, with results normalized to total protein concentration. Statistical analysis was performed using one-way ANOVA to compare stimulation conditions (α = 0.05).

**Results:** Stimulation with LPS and LTA significantly increased MMP-3 levels, with the most pronounced effect observed in LPS-treated DPSCs (p < 0.05). MMP-3 levels remained unchanged in DPSCs stimulated with SM or LR filtrates. None

Continued >

of the bacterial by-products, including LPS, LTA, SM, and LR, induced significant changes in TIMP-4 protein levels ( $p > 0.05$ ).

**Conclusion:** Bacterial by-products differentially regulate MMP-3 expression in DPSCs, but TIMP-4 protein levels remained unaffected by any bacterial stimuli. These findings suggest that MMP/TIMP balance in dental pulp may be specifically altered by certain bacterial components during caries progression.

Supported by: University of Iowa College of Dentistry Student Research Program; NIDCR K08 DE029490

### 53. Molecular Profiling of Hertwig's Epithelial Root Sheath Cells



**Madison Bradley**<sup>1</sup>, *F. Shao*<sup>1</sup>, *H. Cao*<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** In a developing tooth, signaling between Hertwig's Epithelial Root Sheath (HERS) and adjacent mesenchyme is believed to control root development. HERS in a mouse first molar develops at around 7 days postnatal (P7). There have been limited studies on HERS, as tissue processing is difficult at this stage. This study aimed to generate transcriptome profiles of HERS cells during tooth root development, and to test different culture media and protocols for long term in-vitro culture of HERS cells.

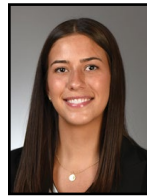
**Methods:** First, we used sectioned P7 mandibles for laser microdissection coupled to RNA-Seq to assess gene expression in HERS and adjacent mesenchyme; a variety of decalcification solutions and durations were tested to optimize preservation of RNA integrity. Second, we used immunofluorescence staining to verify expression of selected genes such as Pitx2 in HERS. Finally, HERS cells from P7 mT/mG Pitx2-Cre mice were captured for 3D cell cultures; several media with different growth/transcription factors were tested to identify optimum conditions for long term in-vitro culture of HERS cells.

**Results:** In preparation for sectioning, laser microdissection and RNA-Seq, optimized decalcification protocol is 20 hours in RNA-Later with 15% EDTA. Immunofluorescence studies confirmed expression of Pitx2 in HERS, substantiating mT/mG Pitx2-Cre lines as an effective method for indicating HERS cells in cultures. Preliminary 3D cell cultures have been successful in growing these cells. Further study into optimized culturing conditions is recommended.

**Conclusion:** These methods will permit future studies into HERS and the mechanisms of root development, with the ultimate goal of building foundational knowledge to develop regenerative therapies.

Supported by: University of Iowa College of Dentistry Student Research Program

### 54. Perception of Urgent Care Dental Curriculum Among Iowa General Dentists



**Madeline Berst**<sup>1</sup>, *S. Lewis*<sup>1</sup>, *C. Pendleton*<sup>1</sup>, *K. Leary*<sup>1</sup>, *A. Gasparoni*<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** To determine what the most important concepts are to teach in the Dental Urgent Care Clinic as part of the curriculum at the University of Iowa College of Dentistry.

**Methods:** A REDcap questionnaire was included in the Iowa Dental Association newsletter inviting all general dentists in the state of Iowa to participate (n=1155). The participants were asked to indicate if topics of a typical dental urgent care appointment should be included in the curriculum. If they answered yes, they were asked to rank the importance of the topic on a scale of 1 (low) to 10 (high). Descriptive information was obtained via statistical analysis that summarized the number and proportion (n, %) of respondents who believed the topic should be included in curriculum, as well as the distribution of ranking importance for each topic (Median, IQR).

**Results:** Response rate was 33 (3%). Twenty topics were surveyed and 82% (n=27) of the respondents worked in private practice with a single practitioner. More than 90% of participants spent less than 25% of their clinic time in dental urgent care. Among the possible topic selections, there was relative uniformity on 8 topics, that all respondents believed should be included in the urgent care curriculum. These topics had a median rank of importance between 7 and 9. The topics include: evaluating medical conditions and medication interactions relative to dental emergency treatment, diagnosing and managing pain and anxiety, along with diagnosing pulpal and associated periapical pathology, periodontal abscesses, cracked teeth, odontogenic pain, alveolar/tooth fractures, and diagnosing teeth as restorable/non-restorable. The other 11 topics ranged from 73-97% of the respondents believing they should be included in curriculum, with ranked importance ranging from 5 to 10.

**Conclusion:** Dentists have varying opinions on what should be included in dental urgent care curriculum, but there was agreement on eight of the topics.

Supported by: University of Iowa College of Dentistry Student Research Program

### 55. Predoctoral Dental Students' Interest in Practice Management at Ulowa



**Reis Schweiner**<sup>1</sup>, *P.C. Damiano*<sup>1</sup>

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**Objective:** Seven competencies have been added to dental school accreditation standards to ensure consistent teaching of practice management. While dental students may not fully grasp what the curriculum should include, identifying their interests and determining the best timing for delivering practice management content can provide valuable insights for both students and faculty.

This project aimed to assess predoctoral dental students' interest in practice management curriculum and to identify factors related to this interest.

**Methods:** All predoctoral students (341) at the University of Iowa College of Dentistry were emailed a survey using the online software Qualtrics (September 2024). This cross-sectional study asked currently enrolled dental students (D1-D4) about their overall interest in practice management and specific interests they have in practice management topics.

**Results:** Approximately thirty six percent of students participated in the survey, with similar response rates across all classes. Among respondents, sixty six percent indicated they are most likely to pursue a career in private practice after graduation. Additionally, ninety four percent expressed moderate to strong interest in required practice management courses, while ninety two percent showed similar levels of interest in elective courses. However, ninety percent reported feeling only somewhat to not very knowledgeable about the practice management curriculum offered while in school. Notably, eighty eight percent of respondents had attended "Lunch and Learn" sessions to enhance their understanding of practice management.

**Conclusion:** The findings highlight a strong interest among predoctoral dental students in acquiring practice management skills, particularly in areas critical to private practice success, suggesting the need for curricular focus on these competencies.

Supported by: University of Iowa College of Dentistry Student Research Program

## 56. Role of Margin Design on Marginal Adaptation and Fracture Strength



**Reid D. Hogan<sup>1</sup>, V. Feitosa<sup>1</sup>, T.S. Porto<sup>1</sup>**

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**Objective:** To evaluate the impact of margin design on the marginal adaptation and fracture strength of crowns fabricated with polymer-infiltrated ceramic network (PICN) and zirconia-reinforced lithium disilicate (ZRLD).

**Methods:** Sixty crowns (n=10) were milled from ZRLD (Vita Suprinity) and PICN (Vita Enamic) CAD/CAM blocks. Each crown was designed from 3D-printed teeth prepared with one of the three following margin designs: light chamfer, heavy chamfer, or modified shoulder. Dual-cure resin cement was used to cement the crowns. Margins were assessed in six standardized areas at baseline with digital scanner (Omniscam, Sirona) and microscope. A score was assigned to each segment based on marginal adaptation (0=closed, 1=open 0.01-0.50mm, 2=open 0.51mm-1.0mm, 3=open >1.0mm). Crowns were then subjected to 500,000 mechanical load cycles to simulate masticatory forces. After cycling, each marginal segment was re-evaluated and rescored. Crowns were then loaded until catastrophic failure to obtain fracture strength (N). Three-way ANOVA was used to statistically analyze marginal adaptation whereas two-way ANOVA was used for fracture strength. After, both were analyzed with Tukey's test ( $\alpha=5\%$ ).

**Results:** All crowns survived the mechanical cycling. After cycling, all margins were significantly more open than before

cycling ( $p<0.001$ ). There was no difference between PICN and ZRLD materials ( $p=0.49$ ) on marginal adaptation. For PICN, more open margins were attained with heavy chamfer than with light chamfer ( $p=0.03$ ). PICN showed no difference ( $p=0.883$ ) in fracture strength among margin designs. Light chamfer in ZRLD crowns provided significantly lower fracture strength compared to other margin designs ( $p<0.001$ ). Light chamfer attained similar fracture strength for both ceramics ( $p=0.589$ ). However, ZRLD was superior to PICN for the other two margin designs ( $p<0.001$ ).

**Conclusion:** Within the limitations of this study, we can conclude that light chamfer finishing margins are preferable to PICN rather than heavy chamfer. Nevertheless, light chamfer may provide lower fracture strength for ZRLD ceramics.

Supported by: University of Iowa College of Dentistry Student Research Program

## 57. Student Perceptions of a Data-Informed Strategy to Reduce Food Insecurity



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**Objective:** Food insecurity (FI) is prevalent among post-secondary students including dental students and is associated with negative academic and health outcomes. In 2023 we conducted a study to identify potential

strategies to reduce FI among dental students. We have since piloted one of these strategies in Iowa's College of Dentistry, and our objective was to evaluate dental students' perception of the strategy.

**Methods:** In response to student-suggested grab-and-go breakfast and lunch items, we introduced a Snack Program Resource in the Student Lounge, featuring perishable (e.g., yogurt) and non-perishable (e.g., granola bars) items. An email was sent to all dental students to inform them of the resource. Approximately six weeks post-implementation, a survey was sent to all dental students (n=333) to assess demographics, food security status, perceptions of the Snack Program, preferred items, usage frequency, and barriers to access. The survey was open for three weeks, and participants received a \$15 grocery gift card as compensation.

**Results:** Of the 156 respondents, 58 or 37% identified as FI. Mean age of respondents was  $24.7 \pm 3.4$  years with 60% female and 82% White. Utilization of the Snack Program Resource showed that 45% never used it, 46% used it 1-2 times per week, 8% 3-5 times per week, and 1% 6-10 times per week. The most preferred items included cheese sticks, protein bars, and mixed nuts. Primary usage times were at lunchtime (33%) and mid-late afternoon (37%). Reasons for not using the resource included not needing it, disliking the choices, being unaware, and being busy. Students appreciated the resource, provided favorable feedback, and offered suggestions for improvement (i.e., greater volume and variety, additional locations).

**Conclusion:** The Snack Resource Program is being used, and students shared ideas to further meet their needs.

Supported by: M.W. Finkelstein Teaching Professorship; University of Iowa College of Dentistry Student Research Program



## 58. A Retrospective Study of Incomplete Primary Root Canal Treatments



**Zachary A. Graham**<sup>1</sup>, F. Qian<sup>1</sup>, C. Hogden<sup>1</sup>

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**Objective:** Patients may fail to complete primary orthograde root canal treatment (RCT) for various reasons. The disease and patient-based outcomes associated with this treatment have not been extensively studied. This study investigated tooth-specific and patient characteristics linked to RCT initiation without completion in an academic setting.

**Methods:** Demographic and clinical data were obtained from axiUm at the University of Iowa College of Dentistry (UICoD) for patients treated between July 2009 and July 2022.

**Results:** A total of 599 subjects (one tooth per subject) met the inclusion criteria. The cohort was predominately female (67.8%), mostly white (70.6%), lived within 60 miles of UICoD (64.3%), and were enrolled in the state-sponsored Dental Wellness Plan insurance (55.4%). Of the 599 teeth, 65.8% had incomplete RCT, 19% were not completed due to non-restorability, 5.5% were extracted for unknown reasons, and 4% were extracted due to patient preference following treatment initiation. At the time of the patient's most recent visit to the UICoD (750.97±1,086.92 days), 38.6% of teeth remained in the mouth, while 37.9% had been extracted or were planned for extraction (23.5%). Pulp necrosis (43.3%) and symptomatic apical periodontitis (58%) were the most common pulpal and periapical diagnoses associated with incomplete RCT. Nearly 62% of teeth with incomplete RCT were molars, and over half (54.4%) were seen in the pre-doctoral clinic.

**Conclusion:** Teeth with pain at diagnosis, molars, and limited clinician experience may increase the risk of patients failing to complete treatment.

Supported by: University of Iowa College of Dentistry Student Research Program

## 59. Alumni Practice Tendencies for Patients with Special Healthcare Needs



**Casey P. Delaney**<sup>1</sup>, J. Hartshorn<sup>1</sup>, M.R. McQuistan<sup>1</sup>

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**Objective:** Access to adequate dental care is a significant issue, particularly for individuals with special healthcare needs (SHCN).

Research shows that many dentists hesitate to treat these patients due to inadequate training. The University of Iowa College of Dentistry established the Geriatric and Special Needs Clinic (GSNC) to enhance dental students' clinical experience with SHCN patients. This study aims to assess how clinical experience with SHCN patients affects practitioner comfort and practices after graduation.

**Methods:** A survey was constructed on RedCap, a secure database approved by the University of Iowa, that included sections such as demographics, education, and practice tendencies regarding SHCN patients. The anonymous survey was distributed to University of Iowa College of Dentistry

alumni that graduated from 2013-2022. Alumni were divided into two groups: a control group, which did not receive GSNC training, and a study group, which did. Data was analyzed between the groups to access the relationship between clinical experience treating patients with SHCN and provider comfort and practice tendencies.

**Results:** There was a total of 35 surveys completed, indicating a 6% response rate. Among the alumni that received GSNC training, 72% thought their dental school education prepared them well or somewhat well in treating patients with SHCN. All respondents agreed to being comfortable treating patients with mild autism, mild intellectual disabilities, and wheelchair bound patients. In contrast, 36% of alumni disagreed with feeling comfortable treating patients with severe intellectual disabilities or severe autism. Finally, 50% of alumni cited low financial compensation and 43% reported increased treatment time as barriers to seeing SHCN patients.

**Conclusion:** Our findings indicate that while alumni thought their education prepared them to treat SHCN patients, additional factors such as condition severity, low reimbursement, and increased treatment time impacted their decision to treat this population.

Supported by: University of Iowa College of Dentistry Student Research Program

## 60. Exploring Mucoadhesive Properties: Buccal Films Dissolution and Stability



**Jacquelyn S. Yeh**<sup>1</sup>, A. Greenwood<sup>1</sup>

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**Objective:** This study aims to develop and evaluate various experimental buccal films for potential oral applications. The goal is to investigate how different polymer compositions and pH modifications impact the films' adhesion, dissolution, and stability in saliva-like conditions.

**Methods:** Multiple prototypes were prepared by varying the concentrations of polymers and other components across four batches. Batch A contained NaOH and a gelling agent, Batch B included NaOH but no gelling agent, Batch C included the gelling agent but no NaOH, and Batch D had neither NaOH nor a gelling agent. These batches were assessed for stability and dissolution in saliva-like solutions, with pH levels also analyzed. The study explored challenges like maintaining stability during storage and optimizing pH.

**Results:** The prototype films exhibited distinct variations in adhesion and dissolution profiles based on their composition. Films without NaOH and gelling agents demonstrated optimal adhesion, flexibility, and prolonged retention on mucosal surfaces. They dissolved gradually, ensuring sustained moisture delivery, and maintained a stable pH suitable for oral applications. These findings suggest that such films could offer comfort and functionality for individuals with xerostomia. Further experiments are needed to confirm these results and refine the formulations.

**Conclusion:** This research demonstrates the potential of buccal mucoadhesive films for future oral applications, with specific focus on balancing adhesion, dissolution, and stability. Further research will focus on refining these formulations to enhance their therapeutic potential and explore clinical

applications. The findings provide a foundation for continued research into patient-friendly solutions for oral healthcare.

Supported by: University of Iowa College of Dentistry Student Research Program

## 61. Exploring the Relationship Between Stress, Diet, Hygiene, and Caries Incidence



**Garry Emmons<sup>1</sup>, H. Steinkamp<sup>1</sup>**

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** Chronic exposure to early-life stressors, known as adverse childhood experiences (ACEs), can disrupt brain development and neural pathways, leading to long-term physical and behavioral health

issues. This phenomenon, termed “toxic stress,” is partly mediated by elevated cortisol levels and is associated with adverse outcomes such as immune dysfunction, heart disease, obesity, mental health disorders, and premature death. Studies suggest that increased salivary cortisol may correlate with higher levels of cariogenic bacteria and dental caries in children. However, research exploring the relationship between stress, hygiene, and caries incidence remains limited.

**Methods:** This study aims to investigate the association between stress, caries, and hygiene in children aged 2–6 undergoing elective surgical procedures at the University of Iowa Stead Family Children’s Hospital. Legal guardians will complete a questionnaire covering topics such as systemic health, oral hygiene habits, diet, socioeconomic status, mental health, and family dynamics. A dental examination will be performed on the children to determine DMFS and plaque levels.

**Conclusion:** This study aims to provide valuable insights into the effects of stress, caries, and hygiene on the oral health of children aged 2–6 undergoing minor surgical procedures at the University of Iowa Stead Family Children’s Hospital. By integrating guardian-completed questionnaires on health, oral hygiene, diet, socioeconomic status, mental health, and family dynamics, this research seeks to deepen the understanding of the complex interplay between stress and oral health. Ultimately, it aims to uncover how adverse childhood experiences and toxic stress contribute to oral health disparities.

## 62. Food Insecurity and Caries: Investigating At-Risk Dental Patients



**Carter Coppinger<sup>1</sup>, C.P. Delaney<sup>1</sup>, O. Rysavy<sup>1</sup>, J.J. Warren<sup>1</sup>, T.A. Marshall<sup>1</sup>**

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** This chart review investigated factors related to food insecurity and the relationship between food insecurity and the prevalence of dental caries using data from a

Caries Risk Assessments (CRA) tool.

**Methods:** This chart review included patients attending the University of Iowa Dental Clinics during June 1, 2021, through

May 31, 2024, who completed a caries risk assessment. Food insecurity was assessed through responses to 2 screening questions and categorized into a binary variable. The relationship between food insecurity and independent variables (age, gender, insurance status, BMI as well as select dietary and health status variable) was analyzed using Fisher’s Exact tests. Dental caries was assessed based on planned dental caries treatments, and its relationship to food insecurity was analyzed with bivariate analyses, using Fisher’s exact test and univariable logistic regression for explanatory variables. Multivariable logistic regression models were developed for basic, advanced, and all caries treatments, employing stepwise AIC selection to determine significant predictors.

**Results:** The sample included 4,594 participants, with 9.5% reporting food insecurity. Those with food insecurity were significantly more likely to have public insurance, eat unstructured meals, consume sugar-sweetened beverages and have a diagnosed mental health condition.

Logistic regression found that food insecurity was associated with an increased likelihood of caries (all caries), although this was not statistically significant (OR: 1.25, p=0.073). Significant predictors for all caries treatments included frequent snack consumption (OR: 1.33, p=0.002), sugary drink intake (OR: 1.25, p=0.012), higher BMI (OR: 1.02, p<0.001) and mental health diagnoses (OR: 1.18, p=0.031) Logistic regression models for basic and advanced caries were similar; interestingly, food insecurity was not identified as a significant predictor for advanced caries treatments.

**Conclusion:** While food insecurity may increase the likelihood of dental caries treatment, frequent snack and sugary drink consumption, higher BMI, and mental health issues are stronger predictors.

Supported by: University of Iowa College of Dentistry Student Research Program

## 63. Policy Trends in Annual Benefit Maximums State Medicaid Dental Programs



**Claire C. Kettle<sup>1</sup>, J.C. Reynolds<sup>1</sup>**

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**Objective:** Annual Benefit Maximums (ABMs) are the maximum amount a dental plan will pay for a member’s dental care each year. ABMs are ubiquitous among private dental plans and are increasingly used in state Medicaid

programs for adults. ABMs have the potential to restrict access to care for Medicaid beneficiaries whose treatment needs exceed their ABM. The objectives of this study were to 1) examine trends in the presence and amount of ABMs in adult Medicaid dental programs and 2) explore associations between ABM use and other state characteristics.

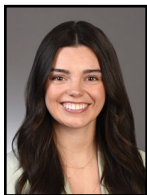
**Methods:** Grey and peer-reviewed literature were searched to collect ABM policy changes as well as other state characteristics. Independent variables were categorized into four domains: Medicaid Policies, Medicaid Population and Spending, Dental Care Access and Oral Health Status, and State Demographic and Geographic characteristics. Bivariate analyses examined associations between the presence of an ABM in 2022 and other state characteristics.

**Results:** The number of states with adult dental Medicaid ABMs increased from two in 2003 to 15 in 2022. The average ABM amount increased from \$538 in 2003 (n=2) to \$1388 in 2022 (n=15). ABM presence was associated with Medicaid reimbursement for children; states with an ABM had higher Medicaid reimbursement than states without an ABM (q=0.034).

**Conclusion:** There was a substantial increase in state Medicaid dental programs adopting ABMs in the last 20 years, and ABM amounts have increased. More research is needed to understand the implications of these trends on dental care access for Medicaid beneficiaries.

Supported by: University of Iowa College of Dentistry Student Research Program

## 64. Pro- and Anti-Fluoride Messaging on TikTok: Sources and Engagement



**Annel Lueth<sup>1</sup>, L.B. Young<sup>1</sup>**

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** TikTok has become a prominent platform for disseminating health-related content, including dental care practices, but the accuracy of such information remains uncertain. Fluoride, a key component in oral

health, is a topic of controversy on social media, making it crucial to assess how TikTok content aligns with scientific evidence. This study aims to evaluate TikTok creators' descriptions of fluoride and identify message characteristics potentially influencing user perceptions of fluoride.

**Methods:** The first 120 TikTok videos were selected that met the search criteria: provided by TikTok in response to the search string, "fluoride vs. no fluoride"; publicly accessible; and English-language content. Videos were viewed and coded on the following variables: message content; source; fluoride perspective; editing style; length; view rate (views/month); and interaction index (likes/views). Analyses included descriptive statistics, Pearson Chi square, t-tests, and ANOVA.

**Results:** Only 22.5% of the messaging was evidence-based and only 40.0% was pro-fluoride, even though 54.1% of the sources were health professionals. Evidence-based content was significantly more likely to be produced by health professionals ( $X^2 = 7.18$ ,  $df = 2$ ,  $p = 0.028$ ), carry a pro-fluoride message ( $X^2 = 11.91$ ,  $df = 2$ ,  $p = 0.003$ ), and be longer ( $t = 2.16$ ,  $df = 118$ ,  $p < .001$ ). Content type did not significantly differ in editing style, view rate or interaction index.

**Conclusion:** TikTok's videos include pro-fluoride and evidence-based content from health professionals, but these are not more popular or engaging than the majority of videos, which are not pro-fluoride and not evidence-based.

Supported by: University of Iowa College of Dentistry Student Research Program

## 65. University of Iowa Dental Student Debt and Career Path Implications



**Mallory Obenauf<sup>1</sup>, P.C. Damiano<sup>1</sup>**

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**Objective:** The Resident Education Deferred Interest Act was introduced into the US Congress in 2023 to assist dental residents with educational debt by allowing them to defer their loan repayments with no interest accrual, until they complete their residency. There is little evidence to date, however, of the effect of student loan debt on dental student's choice to do a residency, and more broadly, the impact of student loan debt on other career choices after graduation.

The primary aims of this study are to: 1) investigate how student loan debt influences UI dental students' practice decisions following dental school, and 2) the potential impact that the ability to defer student loan repayment during postgraduate residency training programs might have on whether to do a residency and/or the type of residency they may choose.

**Methods:** An online survey was administered via Qualtrics to all currently enrolled dental students at the University of Iowa during fall 2023-2024. 121 students responded.

**Results:** About one in four students were interested in applying for a specialty program. Over half of all students indicated that their educational debt made it less likely that they would apply for a dental specialty-this was true for 82% of those without a current interest in a dental residency. Future analyses will focus on the factors related to educational debt as it relates to interest in a dental specialty.

**Conclusion:** Results of the study are still being analyzed. These results will inform policy makers at both the state and federal level of the impact that deferring dental student loan payments may have on a student's decision to pursue a residency program.

Supported by: University of Iowa College of Dentistry Student Research Program; M.W. Finkelstein Teaching Professorship

## 66. Poverty Simulations: Do Personal Experiences and Empathy Influence Learning?



**L. Brendan Young<sup>1</sup>, M.R. McQuistan<sup>1</sup>, C. Pendleton<sup>1</sup>**

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**Objective:** Dental schools use poverty simulations to cultivate empathy for patients facing financial barriers. Research indicates simulations improve attitudes toward poverty, but little research explicitly measures empathy. This study explored associations among personal poverty experiences, empathy, and poverty attitudes before and after a poverty simulation.

**Methods:** Dental students (N = 169) completed anonymous surveys at baseline (T1), post-simulation (T2), and at 2-month follow-up (T3). Surveys included personal poverty experiences, a poverty attitudes measure, and the Interpersonal Reactivity



Index (IRI), measuring empathy. Analysis included paired t-tests, Spearman and Pearson correlations, and quantile regression.

**Results:** Poverty attitude scores increased at T2 by 9.4 points ( $p < .001$ ), and at T3 remained 7.9 points ( $p < .001$ ) higher than baseline. However, the IRI dropped 1.8 points ( $p < .003$ ) at T2, and remained 2.4 points ( $p < .001$ ) lower at T3. Personal poverty experiences were associated only with poverty attitudes at T1 ( $r = 0.16, p < .05$ ). Baseline IRI was not associated with baseline poverty attitudes, but was associated at T2 ( $r = 0.47, p < .001$ ) and T3 ( $r = 0.39, p < .001$ ). For every 1-point increase in baseline IRI, poverty attitudes increased 0.15 (0.05, 0.26) points for the 0.25 quantile ( $p < 0.005$ ) and 0.98 (0.28, 1.7) for the 0.90 quantile ( $p < 0.006$ ). The T1 to T3 change was significant only for the IRI's 0.90 quantile: for every 1-point increase in baseline IRI, poverty attitudes changed 0.94 (0.33, 1.5) points ( $p < 0.003$ ).

**Conclusion:** Following a poverty simulation, poverty attitudes improve, but empathy declines. Personal poverty experiences are not associated with empathy or post-simulation attitudes. Baseline empathy is associated with higher post-simulation poverty attitudes, but with changes in poverty attitudes only for those in the 0.25 or 0.90 quantile. Poverty simulations may be more effective for students already higher in empathy.

## 67. Poverty Simulations: Poverty Attitude Changes and Poverty Simulation Evaluations



**Michelle R. McQuistan**<sup>1</sup>, L.B. Young<sup>1</sup>, C. Pendleton<sup>1</sup>

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**Objective:** Poverty simulations are commonly used to enhance attitudes toward patients facing financial barriers. Research indicates significant improvement in average attitudes

toward those in poverty following simulations, but little research has considered whether students benefit uniformly. The objective of this study was to measure the variability in poverty attitude change following poverty simulations and to determine whether any change is associated with evaluations of the simulation.

**Methods:** Third-year dental students participating in a poverty simulation in 2022 and 2023 were surveyed at baseline (T1), post-simulation (T2), and 2-month follow-up (T3). Poverty attitudes reflect Likert-scale agreement with 20 statements (1-5 points each), addressing personal responsibility and public policies. Simulation evaluations were measured at T2 and T3 by agreement with 8 statements addressing the simulation's usefulness and impact on patient care. Data analysis relied on descriptive statistics, paired-samples t-tests, and Pearson correlations.

**Results:** Students' ( $N = 169$ ) average poverty attitudes were 9.4 points higher at T2 than T1 ( $p < .001$ ) and 7.9 points higher at T3 than T1 ( $p < .001$ ). Among these, however, 21.7% reported lower attitude scores at T2 and 34.6% reported lower attitude scores at T3, with a 4-point median decline from T1 in each group. Baseline poverty attitudes did not predict evaluations, but T2 poverty attitudes correlated with T2 ( $r = 0.33$ ) and T3 ( $r = 0.45$ ) evaluations. Also, T3 poverty attitudes were

correlated with T2 evaluations ( $r = 0.35$ ) and T3 ( $r = 0.44$ ) evaluations (all  $p < .001$ ).

**Conclusion:** Poverty simulations enhance poverty attitudes on average, but potentially worsen attitudes among substantial minorities of students. Those with worse attitudes toward poverty are likely to value the simulation less and may require different teaching strategies.

## 68. Medical-Dental Integration at Federally Qualified Health Centers: Facilitators and Barriers



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<sup>1</sup>University of Iowa, Iowa City, IA; <sup>2,20</sup>National Network for Oral Health Access, Denver, CO

**Objective:** To evaluate the status of Medical-Dental Integration (MDI) at Federally Qualified Health Centers (FQHCs) by identifying barriers and facilitators to implementation.

**Methods:** An online survey was distributed in November 2023 to medical and dental leadership at FQHCs via the National Network of Oral Health Access listserv. Respondents selected the top three barriers and facilitators to MDI implementation. Descriptive and bivariate analyses were conducted to compare perceptions between medical and dental clinics regarding factors that enhance or hinder MDI implementation.

**Results:** There were a total of 31 medical and 180 dental respondents. All medical and 95% of dental respondents reported some form of MDI at their FQHCs. The three most common facilitators were executive leadership support (52% medical and 63% dental respondents), clinical staff willingness to perform MDI services (34% and 44%), and proximity between medical and dental clinics (32% and 30%). The most common barriers were inadequate financial incentives for MDI (22% and 26%), referral process challenges (24% and 19%), and limited time for MDI services during patient visits (22% and 19%). No significant differences were found between medical and dental clinics regarding these factors.

**Conclusion:** Over half of the medical and dental respondents cited leadership support as a top MDI facilitator. The lack of significant differences in barriers and facilitators suggests these issues universally impact MDI implementation. Future research should explore best practices to overcome challenges and strengthen MDI services at FQHCs.

Supported by: Delta Dental of Iowa Smile Grant; HRSA D88 HP37557

## 69. Oral Health Services by FQHC Providers Regarding Early Childhood Caries



**Asana Mohamad**<sup>1</sup>, F. Qian<sup>1</sup>, K. Leary<sup>1</sup>, L.B. Young<sup>1</sup>, M.R. McQuistan<sup>1</sup>

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**Objective:** Early childhood caries (ECC) is a preventable, common condition that causes pain and infection if untreated. Prevention of ECC relies on preventive oral health services (OHS). This study identifies variables associated with federally qualified health center (FQHC) medical providers' provision of OHS and confidence in delivering these services.

**Methods:** A 62-item online survey was developed and distributed to FQHC medical providers nationwide. Provided OHS scores were summed and dichotomized as "few"=0-4 vs. "many"=5-6. Confidence in providing OHS was dichotomized as "more confident" vs. "less confident." Descriptive and bivariate analyses were conducted ( $\alpha=0.05$ ).

**Results:** Eighty surveys were received. Respondents included: Nursing Assistants/Medical Assistants-25%, Nurse Practitioners (NPs)/Physician Assistants (PAs)/Assistant Physicians (APs)-29%, Physicians-30%, and Registered Nurses (RNs)/Licensed Practical Nurses (LPNs)-16%. Sixty-seven percent of respondents had oral health training. Forty-three percent of respondents provided few OHS, while 57% provided many. OHS and confidence in providing OHS varied by clinical role. NPs/PAs/APs and Physicians were more likely than Nursing/Medical Assistants and RNs/LPNs to provide OHS (87% and 86% vs 5% and 33%;  $p < 0.001$ ) and express more confidence in doing so (55% and 57% vs 11% and 8%;  $p < 0.001$ ). Providers with oral health training were more likely than those without training to provide oral health education (77% vs. 55%;  $p=0.041$ ) and evaluate children's teeth (66% vs. 42%;  $p=0.046$ ).

**Conclusion:** The provision of OHS and confidence varied among providers based on their clinical role and oral health training. Further research is needed to increase OHS provided by non-dental providers.

Supported by: HRSA D88 HP37557, James S. and Janice I. Wefel Memorial Research Award

## 70. Impact of Ridge Height and Methods on Mandibular Denture Duplication



**Yuli Gonzalez**<sup>1</sup>, S.H. Cho<sup>1</sup>, T. El Kerdani<sup>1</sup>, S.M. Dabdoub<sup>1</sup>

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**Objective:** The accuracy of complete denture duplication is crucial for maintaining the clinical functionality and comfort of mandibular dentures. Ridge height variations and the choice of duplication methods may influence the precision of the duplicated dentures. With advancements in intraoral scanning technologies, understanding their role in achieving accurate duplicates is essential.

**Methods:** This study evaluated the effect of different ridge heights (low, medium, and high) and two intraoral scanning

systems (TRIOS 5 and PrimeScan) on the accuracy of mandibular complete denture duplication. A total of 3 mandibular dentures representing different mandibular ridge heights were designed using Exocad software and fabricated using the SprintRay 3D printer. The reference scans were performed using a laboratory scanner (Ceramill Map 600), ensuring high-resolution baseline data. Each denture was scanned again using both TRIOS 5 and PrimeScan to generate digital models. Trueness and precision were measured by comparing the duplicated scans to the reference scan using Geo Magic software. Analysis focused on four areas: the teeth, cameo surface, intaglio surface, and the overall complete denture. Deviations were analyzed using 3D analysis software to assess linear and volumetric differences.

**Conclusion:** Ridge height plays a critical role in the precision of mandibular denture duplication. The use of Exocad software and SprintRay printing ensured standardized denture fabrication, while the Ceramill Map 600 provided robust reference scans. Type of scanners significantly influenced the trueness and precision values.

## 71. Biomarker Identification for Oral Cancer Prognosis via Multi-Omics Analysis



**Phuong Nguyen**<sup>1</sup>, J. Kim<sup>1</sup>, X.J. Xie<sup>1</sup>, E. Zeng<sup>1</sup>

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**Objective:** Oral squamous cell carcinoma (OSCC) has a high mortality rate. Advances in high throughput sequencing now allow for integrated multi-omics analysis, providing deeper insights into disease mechanisms. This study aimed to identify potential biomarkers using network-based integrative analysis and paired-gene survival analysis.

**Methods:** Data from 318 head and neck cancer samples (304 cancer, 14 normal) were obtained from the TCGA data portal. Common significant genes from differential gene expression (DEG) and methylation analyses (DMP) were used to construct gene network with iNETgrate, identifying gene modules. Module significance was validated through gene set enrichment analysis (GSEA). Genes were integrated with mutation data for single-gene and paired-gene survival analyses.

**Results:** DEG analysis identified 5,459 significant genes (2,979 upregulated, 2,480 downregulated), while DMP analysis revealed 73,001 significant sites (37,767 hypermethylated, 35,234 hypomethylated). 3,489 common genes formed the fused network, clustered into 11 modules. GSEA of these modules highlighted cancer-related pathways such as WNT signaling and NOTCH signaling. Single-gene survival analysis identified 60 significant genes, while paired-gene analysis revealed 106,804 significant pairs. By examining SNPs of genes selected from the top 10 significant pairs based on paired-gene survival analysis, we identified 9 SNPs across 8 genes associated with the low-survival-rate group and 13 SNPs across 8 genes associated with the high-survival-rate group. However, single-gene survival analysis on these genes identified 7 mutations across 5 genes in the low-survival-rate group and 7 mutations across 4 genes in the high-survival-rate group, highlighting key gene interactions that affect patient outcomes.

**Conclusion:** This study combines network-based analysis and pairwise gene interactions to deepen understanding of cancer biology. Integrating gene expression and methylation data identified key gene modules associated with survival outcomes. The paired-gene survival analysis revealed gene pairs linked to prognosis. These findings highlight potential biomarkers and therapeutic targets for personalized cancer treatment.

## 72. Microbiome Simulation Using MCMC Sampling



**Yiyang Shen**<sup>1</sup>, S. Rajaram<sup>1</sup>, P. Singh<sup>1</sup>, E. Zeng<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** Newly developed sequencing technologies have democratized meta-omics sequencing, generating vast amounts of microbiome data and spurring the development

of novel statistical methods to explore the associations between human microbiota and diseases. However, the inherent complexity of microbiome data – characterized by sparsity, overdispersion, and compositionality – makes realistic microbiome data challenging yet pivotal for methods development and benchmarking. Existing approaches including using statistical models (Dirichlet-Multinomial distribution, gamma-hypergeometric model, and generalized gamma distribution) and neural networks (MB-GAN and DeepMicroGen) have shown varying degrees of success. This is partly because these models rely on simplified assumptions of the input microbiome data that limit their performance and fail to capture the nuances of microbiome data. Here, we propose using Markov Chain Monte Carlo (MCMC) sampling methods to simulate realistic microbiome data.

**Methods:** This method circumvents the need for closed-form gene expression distribution approximations, making the framework for benchmarking statistical tools flexible and elegant.

**Conclusion:** The results demonstrated that the simulated microbiome data generated using the MCMC approach closely resemble real microbiome data, outperforming its competitors. This simulation approach provides a robust framework for evaluating various computational methods, including normalization techniques and differential abundance analyses, to establish best practices for microbiome data analysis.

Supported by: University of Iowa Interdisciplinary Program in Informatics

## 73. Optimizing Data Preprocessing for Oral Multi-Omics



**Priyanka Singh**<sup>1</sup>, S. Rajaram<sup>1</sup>, Y. Shen<sup>1</sup>, E. Zeng<sup>1</sup>

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**Objective:** Oral diseases such as periodontitis pose substantial global public health challenges, with emerging research spotlighting the pivotal role of oral microbes

in such conditions. Integrative whole microbiome sequencing

- metagenomics, metatranscriptomics, and metaproteomics, facilitates the exploration of functional dynamics by examining taxonomy, gene expression, pathway and protein level profiles within the microbiome. In this context, understanding and mitigating technical biases in multiomics data through effective normalization and batch effect correction are imperative to yield meaningful biological insights. In this study, we performed a comprehensive comparative analysis of prevalent normalization methods and batch effect correction strategies to determine the most effective technique.

**Methods:** A systematic review was conducted to compare widely used methods such as TMM (Trimmed Mean of M-values), Arcsine, MinMax, Blom, Inverse Rank, Upper Quantile and RLE (Relative Log Expression) present in contemporary microbial data analysis literature. Subsequently, these methods were utilized on standardized, re-analyzed multiomics datasets from the study by Belstrom et al., which were made publicly available. The statistical measures for each dataset were used to evaluate the central tendencies of the datasets. The abundance values from metagenomics, expression profiles from metatranscriptomics, and protein intensity data from metaproteomics were then utilized to identify the most effective normalization and batch effect correction methodologies for oral multiomics data. This process emphasizes the importance of tailored methods for complex data in microbial ecology. Such an approach provides a comprehensive perspective on the temporal dynamics and current technological advances in oral microbiome research. By comparing the outcomes, we pinpointed the most appropriate techniques for oral multi-omics data analyses, highlighting the necessity for specialized approaches to navigate the intricacies of dataset analysis in microbial studies.

**Results:** Our comparative analysis provides crucial insights into optimizing data preprocessing strategies for the accurate interpretation of each type of multiomics data, thereby enhancing our understanding of microbial influences on oral health and disease.

Supported by: James S. and Janice I. Wefel Memorial Research Award

## 74. Interim Therapeutic Restoration Eligibility among Nursing Home Residents with Caries



**Pamela Nwachukwu**<sup>1</sup>, J. Hartshorn<sup>1</sup>, S.M. Levy<sup>1</sup>, T. Heeren<sup>1</sup>, M. Castelaz<sup>1</sup>, J.C. Reynolds<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA

**Objective:** Nursing home residents experience a high burden of dental disease and substantial barriers to dental care. Interim Therapeutic Restorations (ITR), provided by dental hygienists in community-based settings, have the potential to increase access to caries management services for patients faced with these barriers. The aim of this study was to assess the eligibility of ITR treatment for carious teeth among nursing home residents in a state where ITR was not in the scope of practice for hygienists in community-based settings.

**Methods:** Study subjects included 25 dentate nursing home residents who participated in a broader study about a Virtual

Continued >



Dental Home (VDH) demonstration program. Tooth-level presence of untreated caries was assessed. Carious teeth were assessed for ITR eligibility based on lesion and margin accessibility, lesion depth, and restorability. Additionally, data were collected about residents' medical conditions, cooperation, mobility, and urgency of treatment needs.

**Results:** Among subjects (n=25), most were very cooperative (80%), 40% needed help with dental chair transfers, and 28% had urgent treatment needs. Overall, 92% (n=23) (n=133 teeth; mean:5.3; range:0- 15) of subjects had untreated caries. Among subjects with untreated caries, 87% (n=20) (n=80 teeth; mean:3.2; range:0-12) had a lesion that was eligible for ITR based on study criteria. Among the 80 ITR-eligible teeth, 49% (n=39) were anterior teeth, 19% (n=15) were premolars and 32% (n=26) were molars. For teeth that were ineligible for ITR, the most common reasons were inaccessible lesions and lesions that were too large for ITR.

**Conclusion:** Among subjects with untreated caries in this study, the vast majority (87%) (60% of teeth with untreated caries) had at least one tooth that could have been treated with ITR in the nursing home if allowed by state scope of practice. ITR can be used to treat caries and improve access to care for populations with care barriers.

Supported by: Delta Dental of Iowa Foundation

## 75. Reprogramming of Fibroblasts Into Dental Mesenchymal Cells by Transcription Factors



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**Objective:** Interactions between epithelium and mesenchyme give rise to the development of ectodermal organs, like hair and teeth. In the initiation stage of early tooth development, dental mesenchymal cells have been found an essential role. The project aims to screen a combination of transcription factors to induce human fibroblast cells into dental mesenchymal.

**Methods:** There are many dental mesenchymal transcription factors that have been found in odontogenesis. Among them, we designed and produced lentivirus of the genes identified potentially essential in the dental mesenchymal development. We also performed CRISPR experiments to construct transgenic BJ cells, a type of human fibroblast, with fluorescence reporters on two main dental mesenchymal markers, PAX9 and MSX1 (PAX9: EGFP; MSX1: mCherry). To narrow down the candidates, we will then employ the transgenic BJ cells in the screening for the combination of tissue factors.

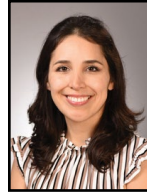
**Results:** Over 20 tissues factors have been marked promising and prepared lentivirus. In constructing PAX9: EGFP; MSX1: mCherry knock-in reporters fibroblasts, we have optimized the procedure for electroporation in BJ cells. AmaxaV solution with program code EN150 and EH100 in the 4D-Nucleofector System was found highest transfection efficiency. We also designed & cloned 3 sgRNAs targeting PAX9 and tested these 3 sgRNAs for CRISPR knock-in efficacy using HEK293 cells.

**Conclusion:** The current results show that BJ cells exhibit appropriate transfection efficiency and could be used as cell line candidate in the project. We have also optimized CRISPR

mediated knock-in protocol with HEK293 cells and will try this protocol in BJ cells. The result of this research will be of value in the tooth regeneration study in the future.

Supported by: NRSA R90 DE024296

## 76. Evaluating Dentists' Perceived Administrative Burden in Iowa's Medicaid Program



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**Objective:** Access to dental services for Medicaid recipients relies substantially on dentists' participation in state Medicaid programs. However, many private practice dentists choose not to participate, often citing administrative challenges such as excessive paperwork, payment delays, and shifting regulatory requirements. While these barriers are well-documented, few studies have examined the perceived administrative burden across dental managed care plans within the same state. This research aims to evaluate the factors associated with Iowa private practice dentists' perceptions of the administrative burden of the Medicaid Dental Wellness Plan (DWP) among dentists participating in the state program's two dental plans.

**Methods:** Secondary data from the 2021 Survey of Iowa Private Practice Dentists resulted in 564 responses from 1,256 mailed surveys (46% response rate), with 497 general dentists included in the final dataset for analysis. The 34-question paper survey collected data on dentist characteristics, practice attributes, and perceptions of administrative burdens. Statistical analyses, including descriptive, bivariate, and multivariable logistic regression, were performed to evaluate relationships between perceived administrative burden and variables such as dentist age, gender, practice busyness, and role within the practice.

**Results:** Preliminary findings show low participation levels among Iowa dentists in the DWP for both managed care organizations, with 27% of dentists accepting new adult patients with Plan 1 and 7% accepting new adult patients with Plan 2. About half of participating dentists in both plans reported the overall administrative burdens as a major burden. Further analyses will evaluate the relationship of the other independent variables with the level of the perceived administrative burden.

**Conclusion:** Administrative burden is a key factor influencing Medicaid dentist participation in Iowa's DWP, with variations observed across managed care plans. Reducing these burdens could encourage better provider participation and optimize access to Medicaid services for enrollees.

## 77. Molecular Mechanisms Underlying Dentin-Pulp Complex Responses to Bacterial By-Products



**Senthilguru Kulanthaivel**<sup>1</sup>, J.A. Banas<sup>1</sup>, O. Rysavy<sup>1</sup>, E. Zeng<sup>1</sup>, D.A. Drake<sup>1</sup>, X.J. Xie<sup>1</sup>, C.M.P. Vidal<sup>1</sup>

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**Objective:** To explore the molecular mechanisms underlying dentin-pulp complex responses to caries progression by studying the gene expression of matrix metalloproteinases (MMP1, 2, 3, 8, 9, 13, and 20) and tissue inhibitors of metalloproteinases (TIMP1, 2, 3, and 4) by dental pulp stem cells (DPSCs) stimulated with bacterial by-products.

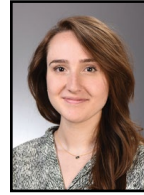
**Methods:** Pure isolates of *Streptococcus mutans* (SM) and *Lactobacillus rhamnosus* (LR) were cultured on blood agar for 48 hours, then transferred to Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2% fetal bovine serum (FBS) and 1% MEM vitamins. After 24 hours, bacterial filtrates were prepared from filter-sterilized supernatants and used to stimulate DPSCs. Lipopolysaccharide (LPS) from *Escherichia coli* and lipoteichoic acid (LTA) from *Bacillus subtilis* served as control bacterial components. DPSCs were treated with/without 20% bacterial filtrates, 10 µg/mL LPS, or 15 µg/mL LTA for 24 hours. Gene expression of MMPs and TIMPs was quantified by RT-qPCR, using GAPDH as the reference gene. Linear mixed models were used to assess statistically significant differences in expression profiles ( $\alpha = 0.05$ ).

**Results:** Both LPS and LTA treatments significantly upregulated MMP1, MMP3, and MMP13 expression compared to controls ( $p < 0.001$ ). LPS specifically increased MMP8 expression ( $p = 0.006$ ), while LTA upregulated MMP2 ( $p < 0.001$ ). LR bacterial filtrate led to significant upregulation of MMP1 and MMP13 and downregulation of MMP8, whereas SM filtrate induced a significant increase in MMP13 expression only ( $p < 0.05$ ). TIMP1 and TIMP3 expression remained unaffected across all treatments, but LPS upregulated TIMP4 ( $p = 0.030$ ) and downregulated TIMP2 ( $p = 0.036$ ). None of the treatments stimulated MMP9 or MMP20 expression in DPSCs.

**Conclusion:** The expression of MMPs and TIMPs in DPSCs is modulated by specific bacterial by-products, with distinct regulatory effects observed. These findings underscore the potential roles of MMPs and TIMPs in the dentin-pulp complex's responses to different bacterial components during caries progression.

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## 78. HIV-Associated Salivary Immune Dysregulation Despite Antiretroviral Therapy



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**Objective:** Despite advances in antiretroviral therapy (ART), Human Immuno-deficiency Virus (HIV) poses a significant public health challenge, particularly in sub-Saharan Africa. HIV infection leads to profound immune dysfunction, increasing susceptibility to both systemic and oral diseases, such as periodontal disease and dental caries. Perinatal HIV transmission, which can occur during pregnancy, delivery, or breastfeeding, raises concerns about immune dysregulation in children. This study investigates whether perinatal HIV exposure, with or without infection, affects salivary immune profiles in children.

**Methods:** Saliva samples were collected from 75 randomly-selected children aged 4–11 at the University of Benin Teaching Hospital in Nigeria. The children were divided into three groups: 5 perinatally-infected with HIV (PHIV+), compared to age- or sex-matched 40 HIV-exposed but uninfected (HEU) and 30 unexposed and uninfected (HUU). Salivary cytokine profiles were analyzed using the Milliplex 48-cytokine panel, which includes markers of inflammation such as IFN- $\gamma$ , IL-6, TNF- $\alpha$ , and IL-8. Kruskal-Wallis nonparametric tests were used to compare cytokine levels across the three groups.

**Results:** The PHIV+ group exhibited significantly elevated levels of 29 out of 48 cytokines associated with increased inflammation and immune cell recruitment, including IFN- $\gamma$ , IL-6, IL-10, Eotaxin, FGF-2, Fractalkine, IL-2, IL-5, IL-12, IL-15, IL-17A, IL-27, RANTES, and TGF- $\alpha$ , when compared to the HEU group. A subset of 15 cytokines, including IL-8, M-CSF, TNF- $\alpha$ , VEGF-A, and IL-22, was significantly higher in the PHIV+ group compared to both the HEU and HUU groups. No significant differences were observed between the HEU and HUU groups.

**Conclusion:** HIV infection in children, rather than perinatal exposure alone, is associated with persistent salivary immune dysregulation, characterized by elevated pro-inflammatory cytokines. This immune dysregulation despite ART, which controls systemic viral load, suggested unresolved localized immune disturbances in the oral environment. Further research is needed to address the long-term oral health effects in children living with HIV.

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*a look back at*  
**Research Day 2024**



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**Initiate Contact Early:** Engaging with the Division of Biostatistics and Computational Biology before data collection is essential. Statisticians can provide guidance on setting up data collection instruments (e.g., Excel sheets or Qualtrics forms) in a format suitable for machine reading and statistical analysis, reducing the amount of time it takes to process research results.

**Active PI Involvement:** Principal Investigators (PIs) play a critical role in approving drafts and making final decisions about the analysis. Their involvement is essential to ensure that the analysis aligns with the study's objectives and the PI's research goals.

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Larger, complex studies may require years of planning, and early collaboration can help optimize the study design and ensure adequate preparation for grant submission.

”  
**Avoid Common Pitfalls:** Failing to consult the Division of Biostatistics and Computational Biology early or collecting data in a non-machine-readable format can lead to significant delays and increased workload for both the division and for the PI. In the past, failure to bring the division into the research process early has resulted in a PI missing a key confounding variable or having a sample size insufficient to support the research conclusions. By addressing these potential issues before they arise, researchers avoid significant roadblocks later in the project.

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Reach out as soon as you have a project idea to leverage the full expertise and resources of the Division of Biostatistics and Computational Biology at the University of Iowa College of Dentistry. Doing so enhances the quality, efficiency, and impact of our research.

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