

IOWA

College of Dentistry
and Dental Clinics

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

71st Annual Meeting

*"Bench, Clinic, and Community:
Iowa Oral Health Research"*

2024





Our Keynote Speaker –

Lawrence A. Tabak, DDS, PhD is the Principal Deputy Director of the National Institutes of Health (NIH). He served as Acting NIH Director from December 20, 2021, to November 8, 2023. He was appointed as the NIH Principal Deputy Director and the Deputy Ethics Counselor in August 2010 following his tenure as Director of the National Institute of Dental and Craniofacial Research from 2000-2010.

Prior to joining NIH, he served as the senior associate dean for research and professor of dentistry and biochemistry and biophysics in the School of Medicine and Dentistry at the University of Rochester in New York. A former NIH MERIT recipient, he has received several honors and awards for his work including election to membership in the Institute of Medicine of the National Academies. He has also received teaching awards for his work with both graduate and medical students.

Keynote Address:

The View From NIH: Many Challenges, Many Opportunities

Dr. Tabak will speak about the NIH and its priorities. He will also provide an overview of his career, and the top ten things he has learned from his leadership career spanning over twenty years.

Dr. Lawrence Tabak,
DDS, PhD

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Our Featured Speakers –



Dr. Huojun Cao,
PhD

Dr. Huojun Cao earned a Ph.D. in Medical Science from Texas A&M Health Science Center in 2011, and he was a postdoctoral fellow in computational biology at Houston Methodist Hospital Research Institute (2012-2016). In 2016, he was appointed assistant professor at the University of Iowa College of Dentistry, where he currently serves as associate professor.

Featured Address:

Molecular Mechanism Controlling the Initiation of Tooth Development Program

Tooth development starts with the formation of the dental lamina, a localized thickened strip within the maxillary and mandibular epithelium. We used Laser Microdissection coupled low-input RNA-seq (LMD-RNA-seq) and single cell multiome-seq to comprehensively identify transcriptional regulatory networks (TRN) controlling the specification of dental lamina from the naïve mandibular epithelium and tooth development. We examined the functional significance of these TFs using loss-of-function models, uncovering the molecular mechanism involving cross-repression of these TFs incl. Pitx2 and Tfp2a/Tfp2b in patterning the mandible’s dorsal-ventral axis and regulating tooth initiation.

Dr. Julie Reynolds is an assistant professor in the Department of Preventive and Community Dentistry and a board-certified specialist in Dental Public Health (DPH). She obtained her DDS (2011) and MS (2013) in DPH from the University of Iowa College of Dentistry, and her areas of research include Medicaid dental policy, quality measurement and improvement in dentistry, dental workforce, and teledentistry.

Featured Address:

Teledentistry Modalities to Improve Dental Care Access and Efficiency

Teledentistry models have the potential to improve dental care access and efficiency, especially for populations that have substantial barriers to care. This presentation will discuss the first models of teledentistry in Iowa, outcomes of preliminary research, and future directions.



Dr. Julie Reynolds
DDS, MS

Iowa Periodontist Examines the Function of PGRPs in Periodontal Disease



Sivaraman Prakasam is associate professor and head of the Department of Periodontics. His research on PGRPs could pave the way for new treatments for periodontitis.

Periodontal disease is caused by infections and inflammation of the gingiva (gums) and bones that support teeth. In some form or another, the disease affects almost 47% of the adults over the age of 30 in the United States and over 70% of adults over the age of 65.

There are strong associations between periodontal disease and various other systemic conditions particularly diabetes. These relationships are well established and often bidirectional. That is, diabetes can increase the risk of periodontal disease and vice versa.

Like many other diseases, prevention tends to be much more effective than treatments. Once established, periodontitis can only be controlled and not cured.

“If we identify and disrupt the disease process early before it becomes particularly destructive, periodontitis is relatively easy to prevent and reverse,” said Sivaraman Prakasam, associate professor and head of the Department of Periodontics.

Prakasam’s work on Peptidoglycan recognition proteins (PGRPs) hopes to add a tool for early identification and treatment of periodontitis that can help dentists and periodontists before periodontitis becomes difficult to treat.

PGRPs are known to recognize essential structures in bacteria.

“This recognition process has evolved over millennia such that their proteins effectively discriminate between good and bad bacteria, and thus helping the body produce an immune response against those bacteria that are harmful to the health of the organism,” explained Prakasam.

For one variety of PGRP, Prakasam and his research team found that those who are periodontally healthy have lower quantities than do those with periodontitis. For another variety of PGRP, they found that those who are periodontally healthy do not have any of that PGRP expressed in samples, while high levels were found in those with periodontitis.

Rather than looking at these proteins merely as a biomarker for periodontitis, Prakasam and his team approached the issue another way.

“Our goal is to better understand what these interactions and signals are doing so that we can develop therapeutic targets for periodontitis,” said Prakasam.

“These proteins and their functions are poorly understood, so we are exploring what these proteins are doing and how they are interacting with the immune response beyond merely recognizing the unhealthy bacteria,” Prakasam explained.

Right now, it isn’t clear which direction the research will take them.

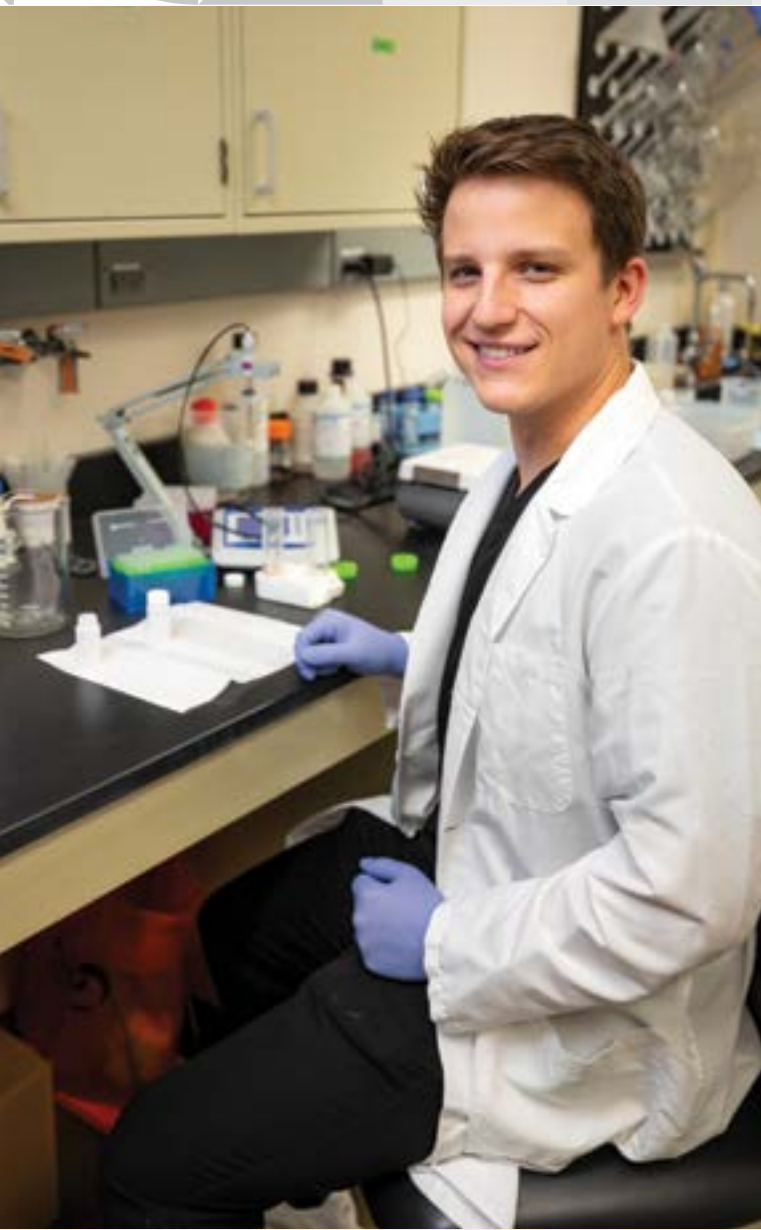
“We know that the proteins are highly increased in saliva and in tissues, but we don’t know why they are increased, We also found in gingiva an increase of these proteins in an unexpected cell type,” said Prakasam.

The National Institutes of Health and the National Institute for Dental and Craniofacial Research awarded Prakasam and his team an R03 grant, an exploratory grant to develop preliminary data for a larger project, to explore the functions of these proteins.

“We used to think that these proteins just recognized good and bad bacteria, but now we are finding important and novel interactions and signaling from these proteins. Our goal is to better understand what these interactions and signals are doing so that we can develop therapeutic targets for periodontitis,” said Prakasam.



Prakasam joined the Iowa faculty in August 2023 as head of the Department of Periodontics. His research is focused on a group of proteins (Peptidoglycan recognition proteins) that are biomarkers for oral inflammatory diseases such as periodontitis, and he has received an exploratory grant from the National Institutes of Health and several private industry and foundation grants in support of this research. He has published over 20 journal articles and has already established strong collaborations with Iowa dental researchers, with many of them contributing to his journal articles.



Student Research Profile: Improving Dry Mouth and Oral Health in Older Populations with Dentures

“Learning how to think critically and appraise research is extremely important—nothing in dentistry is going to stay the same, and we need to be able to take what’s new, evaluate the strength of the research and apply it to practice,” said Colton Curtis, a fourth-year dental student at the University of Iowa.

The Dental Student Research Program at Iowa Dentistry has a long and venerable tradition of instilling these kinds of commitments in its students.

Curtis’ experience over the course of his dental career illustrates this well. He began doing research in his first year, but it took him a while to zero in on the right topic.

“I started doing a literature review with my first idea only to discover that it wasn’t a feasible option for a study,” Curtis explained.

That’s when he began working with Dr. Robert Bowers, assistant professor in the Department of Family Dentistry, and they settled on a research idea.

“The problem was that older patients with a denture often have already demonstrated an increased susceptibility to tooth decay, and they also frequently experience dry mouth,” Colton said, “both of which put them at even higher risk to lose more teeth, which can significantly reduce quality of life for these older adults.”

Curtis and Bowers began to explore MI Paste, a topical product that can protect teeth from decay and promote remineralization. Their idea was for MI Paste to be applied to the underside of a person’s denture,

rather than its labeled use for wiping on the teeth, so that it wouldn’t be washed away as easily via saliva, eating, or drinking, thereby sustaining release of the paste and prolonging the duration of its effect.

“**‘Researching and designing a trial teach you how to make improvements, and you learn so much about evaluating other research,’ Curtis explained. The results were promising as the intervention significantly increased the saliva’s buffering capacity.**”

In the first clinical study, Curtis took baseline measurements of acidity levels and buffering capacity of patients’ saliva, took out their dentures, applied the MI paste under the dentures, placed the dentures back in the patient’s mouth for 15 minutes, and then measured acidity levels and buffering capacity again. Buffering capacity is the degree to which the saliva can resist a significant increase in its acidity level after, for example, drinking an acidic beverage.



Curtis and his research mentor, Robert Bowers, have an effective and productive relationship.

“The study showed us proof of concept—that the paste would release appropriately from under the dentures,” Curtis explained.

Curtis and Bower’s second clinical trial took this proof of concept and designed a daily intervention for patients to use. In this study, patients were asked to apply the MI Paste to the bottom side of their dentures. After wearing them, they would report the subjective assessments related to dry mouth, comfort, and so forth. This study also introduced the use of a second product, Biotene Dry Mouth Gel, to be applied in a similar fashion.

This study adds to the previous laboratory data to show that the off-label use of these products provide patients significant subjective benefits of extended duration, especially for those who suffer from dry mouth.

“This is a really awesome and interesting population to conduct research for,” Curtis said. “It is a very unique group, and there is little research aimed at improving oral health care using therapeutics specifically designed for older adults wearing dentures.”

Curtis plans to continue his education in an orthodontics residency program at Virginia Commonwealth University next year.

He also intends to take the insights he’s gleaned from research into his career.

“Researching and designing a trial teach you how to make improvements, and you learn so much about evaluating other research,” Curtis explained.

Curtis credits his success in research to Bowers’ influence.

“He’s been an incredible mentor, treating me as a colleague while offering guidance and resources well beyond what I expected,” Curtis added.

Supporting Excellence: Iowa Dentistry's Research Support Team

It's a cold wintery day in January, a week before the deadline to submit documents for National Institutes of Health (NIH) grants. The email inboxes and the offices of Iowa Dentistry's grant administration team are flooded with last minute questions and new grant documents as the team gets closer to the deadline. This is one of three NIH submission cycles per year. The research enterprise at Iowa Dentistry is organized around these cycles annually.

“The grant administration team is superb,’ said Xian Jin Xie, associate dean for research in Iowa Dentistry, ‘and our research successes are due in no small part to their on-going efforts to support our faculty and researchers.’”

“Although there is a cyclical process for submitting grants, it really ends up being a yearlong process,” said Ann Lawler, research support manager for the Iowa Institute for Oral Health Research and the lead for the grant administration team in Iowa Dentistry.

Although it was once common for university researchers to be responsible for developing, navigating funding requirements, and submitting their own grant applications, that is no longer the case.

Like most high-level research universities, Iowa Dentistry relies on its grant administration team to help faculty develop their grant applications, ensuring that the applications meet all grant requirements while providing feedback to faculty members and identifying potential weakness in the grant application.



The grant administration team of Maria Bertorello, Mikaela Rhoads, and Ann Lawler frees up faculty to focus on science instead of funding requirements.

"Research Support Team" continued on page 56 ►



Aline Petrin (left), assistant professor in the Department of Orthodontics, is a mentor for students in the Dental Student Research Program. Their research typically focuses on genetic and epigenetic causes of craniofacial anomalies. Third-year dental student Austin Hinkle is mentored by Petrin.

The Dental Student Research Program at Iowa

Iowa Dentistry has one of the most accomplished student research programs in the country, drawing in a large percentage of dental students at Iowa, who go on to make a difference with their student research projects and in their dental careers.

1. Participation

- Each year, there are ~42 dental students who are actively participating in research with funded research projects. That amounts to approximately one-eighth of all dental students at Iowa in any given semester.
- Over the course of a student dental school career, approximately 40% of all dental students at Iowa spend at least one year participating in the program.
- Each year, approximately 36 out of 82 first-year dental students participate in the Dental Student Research Minicourse—Iowa’s introductory course to our student research program.

- Among all institutions in the National Student Research Group, Iowa had the greatest percentage of student members for 2018-2019.

2. Productivity

- For the 2022-2023 academic year, 27 student researchers presented their research at national conferences (e.g., the IADR, the AADOCR, NOHC, AAPD, etc.).
- In 2022-2023, dental student researchers had 8 publications, 3 of which had a dental student as first author.
- In 2022-2023, 4 dental students received AADOCR Travel Bloc Awards, and 2 dental students participated in the AADOCR 411 Competition.
- This year, dental student, Michael Chavez, won the Hinman Outstanding Student Presentation at the Hinman Student Research Symposium at the University of Tennessee.

"Student Research" continued on page 52 ►

Dear Colleagues:

Thank you for your participation in the 71st Anniversary of the University of Iowa College of Dentistry's Iowa Section of the AADOCR on February 13, 2024.

The college's research and discovery mission is critical to the health and vitality of oral health in Iowa. Iowa has long been a leader in dental, oral, and craniofacial research, producing groundbreaking discoveries fundamentally changing the way dentistry and various subfields are performed. That legacy continues, and our discovery research success over the last few years has been remarkable. Our efforts to hire and support excellent established and junior researchers has forged a thriving research enterprise emphasizing collaboration and making a difference in patient's lives.

The research presented today provides a glimpse of these efforts. I want to thank our presenters and the planning committee for their extensive contributions to these efforts.

We are honored to host Dr. Lawrence A. Tabak, the Principal Deputy Director of the National Institutes of Health. Dr. Tabak received his dental degree (Columbia) and PhD and certificate (Endodontics) from the University of New York at Buffalo. Dr. Tabak was a Professor at the University of Rochester Medical Center, New York in Biochemistry & Biophysics where his work involved understanding functional membrane-bound mucin proteins and signal transduction in the innate immune system. Specifically, his current research focuses on the O-glycans tandem repeats and the regulatory roles theses play. He was associate dean for research at Rochester before he came to NIH in 2000 at the Director of the National Institute for Dental and Craniofacial Research (2000-2010) whereupon he became the NIH Principal Deputy Director (2010-present) for the entire NIH. He was the acting NIH Director (2021-2023) before returning to his current position. While it has been a while, Dr. Tabak is an Endodontist by clinical training.

Tabak's research and discovery and his leadership of the NIH has made a profound impact on many of the research projects currently underway at the college. This day is an opportunity for us to showcase how our research and discovery makes 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.

Sincerely,



Clark Stanford, DDS, PhD, MHA
Dean

Dear Fellow Researchers:

The Annual Meeting of the AADOCR displays the wealth of expertise and research at the College of Dentistry and the Iowa Institute for Oral Health Research. Since 2020, the college has achieved unprecedented success in research with a record number of funds awarded and a record number of grant applications. Annually, we are submitting grants for ~\$55 million, and our external funding is approaching \$10 million per year.

This past year our NIH-funded T90/R90 training program was funded again and will continue to support PhD students and postdoctoral researchers. And in collaboration with numerous other colleges across campus, the Oral Cavity Cancer Program is applying for the first Specialized Program of Research Excellence in Oral Cavity Cancer.

Our seed grant program is entering its fourth year. It has successfully helped numerous projects develop the pilot study to secure external grant funding. The college's Clinical/Dental Education Research Initiative Support Program (CRISP) will continue to support clinical and dental education research.

As our research endeavor continues to thrive, our Dental Student Research Program remains one of the best in the country.

This year we are honored to have Dr. Lawrence Tabak, Principal Deputy Director of the National Institutes of Health, as our keynote speaker. Dr. Tabak is a global leader in dental and craniofacial research who has served NIH for many years. Our featured speakers for 2024 are Dr. Huojun Cao, associate professor, and Dr. Julie Reynolds, assistant professor, both in the College of Dentistry.

Thank you for your efforts in support of our research mission. Today is an opportunity for us to showcase the best of our basic, clinical and evidenced-based research studies.

Sincerely,



Xian Jin Xie, PhD
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

Fellow Researchers and Colleagues,

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 71st Annual Meeting, themed "Bench, Clinic, and Community: Iowa Oral Health Research." We are looking forward to sharing groundbreaking research discoveries and fostering new opportunities for collaboration with colleagues.

We are honored to feature Dr. Lawrence A. Tabak, the Principal Deputy Director of the National Institutes of Health (NIH), as our keynote speaker. Dr. Tabak is a world-renowned expert in glycoprotein biosynthesis. Joining him are Iowa's esteemed researchers, Dr. Julie Reynolds, a board-certified specialist in Dental Public Health focusing on dental care access and quality, and Dr. Huojun Cao, an expert in developmental biology, as our featured speakers at our 71st Annual Meeting. 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, including those by numerous graduate students and dental student researchers, will further enrich these areas of study. This collective scholarship represents some of the most exciting developments in dental academia and oral health research. The University of Iowa's commitment to comprehensive research is vividly reflected in the diverse topics covered during this annual meeting, from benchtop to clinical translational to policy research.

As part of the leadership team for the 2024 Meeting of the Iowa Section of the AADOCR, we invite you to join us for this significant event. We trust that you will find it as rewarding and promising as we do. Your participation will undoubtedly contribute to the success of this gathering.

Thank you for being a part of our vibrant community, and we eagerly anticipate the knowledge exchange and networking opportunities that this event will bring.

Sincerely,



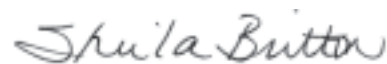
Sukirth Ganesan, BDS, PhD
President, Iowa Section of the AADOCR
Assistant Professor
Department of Periodontics



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



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



The University of Iowa College of Dentistry and Dental Clinics welcomes our presenters from Chongqing Medical University, School of Stomatology, Chongqing, China for the 2023 Annual Meeting of the Iowa Section of the AADR. The UICOD and the CMUSS are excited to extend our past collaborative efforts, paving the way for a healthy, long-term research partnership between these two institutions to advance the exchange of ideas, research, and education, each of which strengthens oral health initiatives here and abroad.

Milestones of Achievement Between Iowa and Chongqing

- The University of Iowa College of Dentistry (UICOD) established a Memorandum of Agreement with Chongqing Medical University School of Stomatology (CMUSS)—To collaborate for the Advancement of Oral Health Research and Scholarship in Oral, Craniofacial and General Health (September 2016).
- Faculty and colleagues from CMUSS presented their research at the 2018 and 2019 Annual Meetings of the Iowa Section of the AADR. This partnership helped strengthen our commitment to the exchange of ideas, research, teaching and training opportunities for all of our faculty and students (February 2018 and February 2019).
- Faculty from the University of Iowa attended the 1st Sino-U.S. Summit Forum of Dental Hospitals for Faculty-to-Faculty Cooperative Exchange. This formal summit included presentations from UICOD and CMUSS faculty (May 2018).
- Since 2018, the UICOD and CMUSS faculty have several joint research projects and collaborations, including:
 1. A joint research project on microRNA-based craniofacial bone regeneration, with Chongqing researchers performing animal experiments that demonstrated the enhanced mandibular bone regeneration using plasmid microRNA-incorporated in modified collagen plugs. Iowa researchers participating in the project came from the labs of Drs. Brad Amendt, Liu Hong and Hongli Sun.
 2. Dr. Yi Shu, D.D.S., Ph.D., is a visiting scholar from Chongqing who is conducting research in Dr. Liu Hong's lab. Dr. Shu is an assistant professor of endodontics at Chongqing Medical University.
 3. Dr. Jin Xie is advising Dr. Tao Chen on clinical research infrastructure as Chongqing Medical University School of Stomatology develops its clinical research center.
- Faculty and colleagues from CMUSS gave poster presentations at previous Iowa Section of the AADOCR Annual Meetings since they were unable to travel to the U.S. (2021-2024).

Program

Iowa Section of the American Association for Dental, Oral, and Craniofacial Research (AADOCR)
71st Annual Meeting, Tuesday, February 13th, 2024

7:30 a.m.	Breakfast (First Floor Link)
Morning addresses will be presented in the Galagan Auditoria	
8:00 a.m.	Welcome Address Dean Clark Stanford
8:10 a.m.	Welcome Address, and Introduction of Featured Speakers Associate Dean for Research, Dr. Jin Xie
8:20 a.m.	Featured Speaker Dr. Huojun Cao <i>"Molecular Mechanism Controlling the Initiation of Tooth Development Program"</i>
8:37 a.m.	Featured Speaker Dr. Julie Reynolds <i>"Teledentistry Modalities to Improve Dental Access and Efficiency "</i>
8:55 a.m.	Keynote Speaker Introduction Dr. Sukirth Ganesan
9:00 a.m.	Keynote Address Dr. Lawrence Tabak <i>"The View From NIH: Many Challenges, Many Opportunities"</i>
9:45 a.m.	Break
10:15 a.m. - 11:45 a.m.	Oral Presentations (Galagan A, Gal. B, Gal. C, W205, W333, W433, S483, & N212 Oral-B classroom)
12:00 p.m. - 1:00 p.m.	Poster Presentations (Iowa Institute for Oral Health Research, Fourth Floor Link, W220A/B)

Abstracts

Presenters are underlined Mentors are *italicized*

Oral Session 1	
1. Genomics and Epigenomics of Twins With Mirror Image Cleft Lip	2. Whole Genome Sequencing of a Family of Autosomal Dominant Macrostomia
 Waheed O. Awotoye ¹ , L. Alves Machado De Paula ¹ , L.T. Hovey ¹ , M. Chimenti ¹ , B.W. Darbro ¹ , E. Zeng ¹ , X.J. Xie ¹ , S.M. Dabdoub ¹ , J. Thomas ¹ , J.C. Murray ¹ , S. Rengasamy Venugopalan ¹ , L.M. Moreno Uribe ¹ , A.L. Petrin ¹ ¹ University of Iowa, Iowa City, IA	 Austin B. Hinkle ¹ , L. Alves Machado De Paula ¹ , L.T. Hovey ¹ , W.O. Awotoye ¹ , M. Chimenti ¹ , B.W. Darbro ¹ , L. Arilho Ribeiro Bicudo ² , S.M. Dabdoub ¹ , T. Peter ¹ , J.C. Murray ¹ , S. Rengasamy Venugopalan ¹ , L.M. Moreno Uribe ¹ , A.L. Petrin ¹ ¹ University of Iowa, Iowa City, IA; ² Universidade Federal de Goiás, GO, Brazil
Objective: Disturbances in the intricate processes that control craniofacial morphogenesis result in birth defects, most common of which are orofacial clefts (OFCs). Non-syndromic cleft lip (nsCL) is one of the phenotypic forms of these OFCs which has a non-random laterality presentation, with the left side being affected twice as often as the right side. This study investigates the etiology of nsCL and the factors contributing to its laterality using a pair of monozygotic twins with mirror-image cleft lip.	Objective: Macrostomia is a rare disorder that can occur in isolation or association with conditions within the Oculoauriculovertebral Spectrum (OAVS), which encompasses a wide variety of anomalies derived from the first and second branchial arches. We present the genetic findings of a three-generation family with multiple members affected with macrosomia, preauricular tags, and ptosis—the first reported instance of autosomal dominant inheritance of this type.
Methods: We conducted whole-genome sequencing (WGS) analyses in a twin pair with mirror image nsCL and their parents to identify etiopathogenic variants. Additionally, DNA-methylome analyses were conducted to identify differential methylation patterns between the mirror-twins as well as a replication in an independent cohort of patients with unilateral cleft lip (left=22; right=17).	Methods: Whole genome sequencing data were generated for the proband, affected father, and unaffected paternal grandmother. We used the Mouse Genomics Informatics Database, gNOMAD, Varsome and Domino Database to filter out common variants and assess pathogenicity and inheritance model of the potential causal mutations. We then verified segregation of the top 10 candidate genes: <i>KCND2</i> , <i>PDGFRA</i> , <i>CASP9</i> , <i>NOCA3</i> , <i>WNT10A</i> , <i>SIX1</i> , <i>MTF1</i> , <i>KDR</i> , <i>LRRK1</i> , and <i>TRIM2</i> in 23 family members. Parent and sibling-based transmission disequilibrium and burden analysis tests explored the effects of candidate genes, and bioinformatic methods investigated gene and phenotype connection.
Results: We identified protein-altering variant in <i>FGF20</i> (p.Ile79Val) within the fibroblast growth factor interacting family domain segregating with the nsCL in the family. Concurrently, DNA-methylome analysis identified differential methylation regions (DMRs) upstream of Zinc-finger transcription factor <i>ZFP57</i> ($\beta > 5\%$). Replication studies confirmed these DMRs, emphasizing their biological significance (p=0.04). Enrichment analysis implicated these DMRs in DNA methylation involved in embryo development (adjusted FDR p-value = 1.3241E-13). Further bioinformatics analysis showed one of these DMRs acting as a binding site for transcription factor AP2A (<i>TFAP2A</i>), a key player in craniofacial development. Interactome analysis also suggested a potential role for <i>ZFP57</i> in left/right axis specification thus emphasizing its significance in cleft laterality.	Results: <i>SIX1</i> , <i>KDR</i> , and <i>PDGFRA</i> were associated with OAV phenotypes. TDT indicated a potential association of <i>SIX1</i> mutations with affected status (p=0.025, p=0.052), while <i>KDR</i> (p=0.180, p=0.069) and <i>PDGFRA</i> (p=0.180, p=0.069) may have similar associations. Burden analysis highlighted <i>SIX1</i> (RC=0.87) and <i>PDGFRA</i> (RC=0.98) as strongly linked to OAVS severity. Sib TDTs identified associations between <i>SIX1</i> and ptosis (p=0.049), and <i>PDGFRA/KDR</i> with ear tags (both p<0.01). Bioinformatic analyses revealed specific pathways connecting <i>SIX1</i> to <i>PDGFRA</i> and <i>KDR</i> to <i>PDGFRA</i> .
Conclusion: This study provides novel insights into the etiology of nsCL and its laterality, suggesting an interplay between etiopathogenic variants and DNA methylation in cleft pathogenesis. Our findings elucidate the intricate mechanisms underlying OFCs development. Understanding these factors may offer insights into the prevention and management of OFCs, alleviating the burden on affected individuals, their families and global health.	Conclusion: This study unveils unique genomic findings in a large pedigree with autosomal dominant inheritance of OAVS phenotypes and narrowed down to three potential candidate genes— <i>SIX1</i> , <i>PDGFRA</i> , and <i>KDR</i> . Future research includes animal studies and a larger cohort to study mutation impacts. Identifying genes involved in OAVS not only provides crucial information to affected families but also serves as a foundation for researchers to further study the condition.
Supported by: NIDCR K01 DE027995.	
Supported by: NIDCR K01 DE027995, R37 DE008559; University of Iowa College of Dentistry Student Research Program.	

3. Whole Genome Sequencing Identifies Pathogenic Variants in Africans With Cleft



Emmanuel T. Aladenika¹, M. Olujitan¹, L. Cook¹⁷⁷, A. Alade¹, W.O. Awotoye¹, L.J. Gowans¹²⁷, M. Eshete²¹³, T. Naicker¹⁴⁵, W.L. Adeyemo¹²⁸, A.M. Oladayo¹, T. Busch¹, A. Visel¹⁷⁷, J.C. Murray¹, P.A. Mossey¹⁵⁶, A.A. Adeyemo¹⁵⁵, A. Butali¹

¹University of Iowa, Iowa City, IA; ¹²⁷Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; ¹²⁸Department of Oral and Maxillofacial Surgery, University of Lagos, Akoka, Lagos, Nigeria; ¹⁴⁵University of KwaZulu-Natal, Durban, South Africa; ¹⁵⁵National Human Genomic Research Institute, Bethesda, MD; ¹⁵⁶Department of Orthodontics, University of Dundee, Dundee, UK; ¹⁷⁷Lawrence Berkeley National Lab, Berkeley, CA, USA.; ²¹³Addis Ababa University, School of Medicine, Department of Surgery, Addis Ababa, Ethiopia.

Objective: The contributions of non-coding regions to the development of orofacial clefts (OFC) have been reported. We investigated variants within craniofacial enhancers that may disrupt transcription factors binding sites, hence, the expression of target craniofacial genes.

Methods: Whole genome sequencing was conducted on 130 African case-parent trios (affected probands and unaffected parents). Variants were called and annotated using GATK. Putative craniofacial enhancers with very rare (MAF <0.1%) and novel variants were filtered using Varseq software. These putative enhancers were intersected with in-vivo validated enhancers. Deleteriousness of variants in intersected enhancers were predicted with a CADD score cutoff of ≥ 15 . Variants predicted by RegulomeDB to affect transcription factor binding were prioritized. Genes within the same TAD as enhancers with these deleterious variants were identified using UCSC genome browser. The contribution of these genes to craniofacial development were assessed with MGI, OMIM and CleftGeneDB. Craniofacial genes in any of the 3 databases were prioritized.

Results: We identified 1795 putative craniofacial enhancers with more than 1 novel or very rare variant. Of these, 175 intersected with in-vivo functionally validated enhancers. Sixty-nine were reported to be active in facial-mesenchyme, branchial-arch, or frontonasal regions. Twenty-five of these 69 enhancers harbor 46 pathogenic variants. Of these 46, RegulomeDB predicted 5 to affect binding of transcription factors involved in craniofacial development. Chr14:53571107-53572814 near *BMP4* has a novel variant. Two very rare variants, rs935218751 (MAF=0.0002412) and rs887296504 (MAF=0.0002416) were in enhancers near *NFIA* and *HS2ST1*; respectively. *BMP4* is a known cleft candidate gene, while *NFIA* and *HS2ST1* have mouse models with craniofacial phenotypes.

Conclusion: Our study suggests that novel variant in an in-vivo validated craniofacial enhancer close to *BMP4* and very rare variants near *NFIA* and *HS2ST1* may affect regulation of these genes and hence contribute to OFC development. Experiments to validate the functionality of these variants are in progress.

Supported by: NIH R01 DE028300.

4. Impact of Secondary Findings on African Cleft Caregivers' Mental Health



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Objective: Whole-genome sequencing (WGS) may unveil secondary findings (SFs) of health significance, intensifying psychological distress faced by caregivers with children affected by orofacial clefts (OFCs). However, little is known about caregiver experiences and the impact of receiving SFs in the context of OFCs in sub-Saharan Africa. Thus, we aimed to explore the impact of SFs on the psychosocial well-being of cleft caregivers in Nigeria.

Methods: Two Focus Group Discussions were conducted with 22 purposively sampled caregivers of children who underwent WGS analysis and attended the cleft clinic at the LUTH, Lagos, Nigeria. We investigated participants' experience regarding genomic testing, knowledge about SFs, psychosocial impact and coping strategies. Audio recordings from discussions were transcribed verbatim and analyzed using content analysis.

Results: The average caregiver age was 34.3 ± 11.3 years, and 19 out of the 22 participants were females. Four themes and thirteen subthemes emerged. These include concern {causes of cleft, acting on SFs and community treatment of affected child}, pervasive burden {childcare practices, financial hardship hindering follow-up on SFs, physical and mental health impact and effect on family unit}, coping strategies {holding on through faith, family support and peer support at cleft clinic} and need for psychological support {coping with cleft diagnosis, associated stigmatization and requirements of caring for a child with cleft}. Although WGS results sparked relief and concerns, caregivers reported that SFs might potentially exacerbate existing stress.

Conclusion: Caregivers faced numerous difficulties, including psychosocial issues, as they cared for a child with a cleft. However, interactions with fellow parents and care providers at the cleft clinic provided vital support and motivation. Thus, promoting caregiver-provider collaboration becomes pivotal in enhancing understanding and utility of testing results. Additionally, it could facilitate connection to essential resources capable of offering much-needed psychosocial support, thus mitigating adverse caregiver experiences often associated with OFCs in resource-constrained settings.

Supported by: NIH R01 DE028300; NIDCR T90 DE023520.

5. Genetic Epidemiology of Cancer Risk in Orofacial Cleft Cases



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Objective: Epidemiological studies have identified an association between orofacial clefts and an increased risk of cancer. While some individual studies suggest a plausible connection between familial cancer risk and orofacial clefts, the existing literature provided conflicting reports on this association. The aim of this study was to assess the prevalence of pathogenic variants within established cancer predisposition genes (CPGs) among families affected by orofacial clefts.

Methods: We filtered for variants within 60 recognized autosomal-dominant CPGs, including BRCA1 and BRCA2, in an African-only whole genome sequencing (WGS) dataset. This dataset included 130 case-parent trios with non-syndromic cleft lip with or without cleft palate (NSCL/P). We prioritized variants predicted to be damaging based on scores from the in-silico predictive tools - CADD, REVEL, SIFT, and PolyPhen2. Our analyses included rare variants (minor allele frequency $\approx 1\%$) that were present in the cases and transmitted from either parent.

Results: We identified 12 rare variants in CPGs, with 13% of probands carrying one or more potentially pathogenic variants in a CPG. Notably, four of these variants, located in SUFU, TSC2, CDH1, and MSH6 genes, were consistently predicted as damaging by all four in-silico tools, and have been associated with hereditary cancer-predisposing syndromes. Additionally, two of these variants, p.Asn1681Asp in TSC2 and p.Asp336Ala in SDHA, were predicted to be likely pathogenic according to the American College of Medical Genetics and Genomics (ACMG) criteria.

Conclusion: Although the etiology of cancer is known to be multifaceted, our findings suggest a possible elevated risk of cancers in individuals with clefts. Nevertheless, these results must be taken with caution. Further studies are essential to gain a more comprehensive understanding of the relationship between clefts and cancer risk.

Supported by: NIH DE28300.

Oral Session 2

6. Fracture Resistance of CAD/CAM Veneers Fabricated With Pre/Fully-Crystallized Lithium Disilicate



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Objective: This study aimed to evaluate the fracture resistance of chairside CAD/CAM veneers fabricated with the traditional two novel pre-crystallized and two novel fully-crystallized lithium disilicate ceramic materials.

Methods: Seventy-five chairside CAD/CAM veneers (15 specimens/group) for maxillary right central incisors were fabricated with different lithium disilicate brands: (1) IPS e.max CAD; (2) Amber Mill; (3) Cerec Tessera; (4) n!ce Straumann; and (5) GC Initial LiSi Block. Restorations were cemented with resin luting cement (Variolink Esthetic, Ivoclar Vivadent) to 3D-printed resin dies. Bonded restorations received 5,000 thermal cycles and then were loaded until fracture. Statistical analysis conducted in this study, included both ANOVA and independent samples t-tests.

Results: Traditional pre-crystallized e.max CAD displayed the highest fracture resistance value (640N), followed by fully-crystallized n!ce Straumann (547 N), pre-crystallized Cerec Tessera (503 N), pre-crystallized Amber Mill (476 N) and the lowest values by fully-crystallized GC Initial LiSi Block (431 N). When comparing the fracture resistance of novel Lithium Disilicate ceramic material to the Emax group, which acted as the control, significant differences were noted. The LiSi Block GC group, in particular, had a considerably higher mean difference values (208.867, $p < .001$, 95% CI [89.63, 328.10]), as did the The Amber Mill group (164.200, $p = .002$, 95% CI [44.96, 283.44]) and CEREC Tessera group (137.533, $p = .016$, 95% CI [18.30, 256.77]). The e.max and N!ce Straumann groups had no statistically significant differences in mean scores (92.933, $p = .198$, 95% CI [-26.30, 212.17]). These findings imply that the clinical performance of novel lithium disilicate veneers varied when compared to the e.max CAD group.

Conclusion: The fracture resistance of chairside CAD/CAM lithium disilicate veneers for maxillary central incisor varies according to the type of ceramic brands. Conventional pre-crystallized e.max CAD displayed higher fracture resistance than novel pre- and fully-crystallized lithium disilicate materials, emphasizing the significance of choosing the right product based on the desired clinical outcome.

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

7. Fracture Resistance of Anterior Crowns With Different Endodontic Access



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Objective: To determine the fracture resistance of computer-aided design and computer-aided manufacturing (CAD-CAM)

lithium disilicate crowns for mandibular right central incisor without and with lingual, incisal and facial endodontic access. It also evaluated the effect of thermocycling on half of the specimens.

Methods: Eighty milled lithium disilicate (e.max CAD, Ivoclar Vivadent) crowns were designed and fabricated with a dental chairside CAD/CAM system (Planmeca Romexis, Planmeca). The restorations were treated with primer system (Monobond Plus, Ivoclar Vivadent) and cemented with resin luting cement (Multilink® Automix, Ivoclar Vivadent) to resin printed dies (Gray Resin V4, FormLabs). Half of the cemented crowns were subjected to 10,000 cycles of thermal cycling between 5 and 55°C. Restorations were divided in groups (n = 10). Restorations were exposed to compressive force using universal testing machine. SEM images of the broken specimens were captured. ANOVA test was used for statistical analysis.

Results: The fractured resistance varies according to the endodontic access location. Crowns with no thermocycling displayed higher values than crowns with thermocycling. Crowns with no access and no thermocycling received the highest values (1218 N) followed by crowns with lingual access with no thermocycling (1187 N), crowns with no access and no thermocycling (1088 N), crowns with facial access and no thermocycling (1023 N), crowns with lingual access and with thermocycling (898 N), crowns with facial access and with thermocycling (893 N), crowns with and the lowest values for crowns with incisal access without (822 N) and with thermocycling (720 N).

Conclusion: The fracture resistance of chairside CAD-CAM mandibular central incisor crown decreases with endodontic access. Crowns with lingual endodontic access displayed higher values than crowns with facial and incisal access.

8. Fracture Resistance of Interim Veneers: CAD/CAM Milled Versus Direct Hand-Made



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Objective: Dental laminate veneer restorations are crucial role preserving enamel and minimizing tooth structure removal during conservative restorative procedures while meeting esthetic and functional requirements. Provisional laminate veneers are essential for pre-evaluating final restorations, preventing dental

or periodontal complications, and ensuring patient comfort and function.

Purpose: This study rigorously evaluates the fracture resistance of two type interim veneer restorations for maxillary central incisors: milled (subtractive Computer-Aided Manufacturing [s-CAM]) versus traditional direct hand-made bis-acryl

Methods: Fifty maxillary right central incisor veneers (25 specimens per group) were fabricated and divided according to the fabrication method: (1) s-CAM milled (Structure CAD, VOCO Dental); and (2) hand-made (Protemp Plus, 3M). The restorations were cemented onto 3D-printed resin dies using temporary cement and subjected to 1,000 cycles of thermal cycling between 5° and 55°C. Subsequently, the restorations were loaded to compressive load until fracture. Scanning electron microscope (SEM) images of the fractured samples were captured. Statistical analysis was performed using the one way ANOVA test and Mann-Whitney test

Results: The fracture resistance of the two groups exhibited significant differences. s-CAM milled interim veneers displayed higher fracture resistance mean values (439.60 N) compared to the traditional method (149.15 N) (p <0.001).

Conclusion: The manufacturing method significantly influences the interim veneer restorations' fracture resistance. s-CAM interim veneer restorations for maxillary central incisors exhibit superior resistance than the traditional method using bis-acryl. Clinicians should consider CAD/CAM milled veneers for scenarios demanding long-term interim restoration and withstanding high occlusal forces.

9. 10MDP Mediated Adherence of RCLA to Zirconia Fracture Mechanics Approach



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Objective: The success of all-ceramic restorations depends on a strong and stable bond to dental hard tissues, achievable by adhesive cementation. 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) is a suitable primer for zirconia-based restorations. Adherence to zirconia imparted by 10-MDP has been investigated with shear and micro-tensile bond strength tests. This study aimed to apply fracture mechanics methodology to investigate the effect of 10-MDP on the adherence of a resin composite luting agent (RCLA) to recently introduced rapid-fired zirconia (RFZ).

Methods: Interfacial fracture toughness (IKIC) was determined with the notchless triangular (NTP) specimen KIC test. Seventy-eight NTP specimens were cut and ground from RFZ blocks (Katana, Kuraray, Japan), followed by rapid firing. The samples were then cut into halves and allocated to three groups, each with a different surface preparation protocol prior to bonding: 1) Control, no treatment; 2) MDP, 5 % 10-MDP ethanol primer; 3) Silane, Bisco Bis-Silane. All samples were bonded with an RCLA (3M RelyX Veneer Cement) and stored in water at 37°C. IKIC was determined after 24 h and 90 d storage. The results were analyzed with an independent samples t-test (α= 0.05).

Scanning electron microscopy fractographic analysis was performed on representative fractured samples from each group.

Results: At 24 h, only the MDP group could be tested [IKIC (1.34 ± 0.40) MPa√m^{1/2}]. Samples from the other two groups were de-bonded before testing. For the MDP group, crack propagation occurred cohesively through the RCLA. After 90 d storage, the IKIC of the MDP group dropped significantly, to (0.88 ± 0.3) MPa√m^{1/2}.

SEM images of fractured surfaces (24 h and 90 d) showed the presence of RCLA on both halves, indicative of cohesive failure within RCLA.

Conclusion: The fracture mechanics analysis confirmed the suitability of MDP as a primer for RFZ. Even though there was a significant decrease in IKIC upon storage, failure always took place cohesively in the RCLA.

Supported by: Kuraray America.

10. Advanced 3D Metal Additive Manufacturing for Dental Substructures



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Objective: Additive Manufacturing has received much attention in dentistry as CAD/CAM produces fixed dental restorations.

Potential benefits of AM mass production of substructures readily printed from digital files include reduced material waste due to the additive process and the ability to replicate intricate features needed for patient-specific restorations. To evaluate the material, mechanical properties, and geometric accuracy of CoCr dental substructures using AM and conventional methods.

Methods: CoCr test bars and 3-unit dental substructures were AM by SLM technology and conventional methods of milling and casting. Test bars were designed using ASTM E290 and underwent 3-point bend testing. Additional testing on test bars includes microstructural analysis, hardness and porcelain adherence. FDP substructures will undergo *in vitro* testing along with the test bars to determine their flexural strength. Thus far, the FDP substructures have been scanned using high-energy and high-resolution micro-CT imaging to evaluate porosities within the FDP substructures and quantify geometric accuracy before mechanical testing. In addition, the micro-CT scans were used to generate 3D digital models of the final alloy substructures for marginal fit comparisons with the ideal FDP model.

Statistical analysis of all data was conducted using one-way ANOVAs and Tukey's multiple comparative tests at a 95% confidence level.

Results: AM test bars exhibited significantly greater flexural strength than cast and milled bars. However, no significant difference was found in alloy stiffness. Micro-CT scans of the FDP substructures revealed greater porosity within cast versus AM and milled samples. All micro-CT models (AM, milled and cast) exhibited surface deviations within 12 μm from the ideal CAD model.

Conclusion: This study will provide essential data to critically assess AM technology for the routine fabrication of dental substructures. Such information will be essential for the future adoption of AM in the Canadian dental manufacturing sector.

Supported by: ADEISS; Western University, Ontario, Canada; Rotsaert Dental Laboratories, Ontario, Canada.

11. Preparation Designs and CAD/CAM Materials Effects on Onlay Fracture Resistance



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Objective: To evaluate the impact of two onlay preparation designs when restored with different CAD/CAM materials on fracture resistance in comparison to sound teeth structure.

Methods: Sixty crack-free, sound human molars (n=12/group) were randomly divided into five groups: C (Control, sound tooth), A-Hyb (Design-A preparation with rounded-shoulder finish line) restored with hybrid nano ceramic block, A-LiSi (Design-A prep with rounded-shoulder finish line) restored with lithium disilicate block, B-Hyb (Design-B preparation with butt-joint finish line) restored with hybrid nano ceramic block, B-LiSi (Design-B prep with butt-joint finish line) restored with lithium disilicate block. The cementation process involves a selective etch-and-rinse on enamel, followed by applying a self-adhesive primer and self-adhesive resin cement. The specimens were thermo-cycled (10,000 cycles, simulating approximately 1 year of aging), followed by mechanical cycling (500,000 cycles at 50 N, positioned 90° with the occlusal surface at a frequency of 1 Hz, simulating approximately 2 years of aging). The fracture resistance of the tooth-restoration system was assessed through a compressive test using a Zwick universal machine. The specimens were subjected to compressive load at a 90° angulation using a 5 mm stainless-steel conical-shaped piston in a compression test apparatus until fracture. Data was statistically analyzed by two-way ANOVA with α = 0.05.

Results: No statistically significant differences were observed among groups considering preparation design (p = 0.972) or materials (p = 0.278) and they were not statistically different than the sound tooth (p > 0.05).

Conclusion: Onlay restorations fabricated with CAD/CAM materials achieved comparable fracture resistance to those of sound teeth when applied with precision and adherence to clinical and manufacturing guidelines regardless of the preparation design and the material selection.

Supported by: Iowa Institute for Oral Health Research; Iowa Health Technology Committee; GC America®.

12. Dental Alumni's Provision of Care to Underserved Populations



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Objective: The objective of this study was to investigate dentists' treatment of underserved populations.

Methods: A survey was developed and mailed to 741 alumni of the University of Iowa College of Dentistry (Classes of 2009-2018). The survey inquired whether they provide care to 13 underserved populations and the reasons underlying their decisions not to treat these patients. It also collected information about the respondents, including demographic information, indebtedness and their comfort in treating underserved populations. Lastly, the survey inquired about alumni's interest in participating in CE courses related to underserved populations. Statistical analysis encompassed descriptive, bivariate, and logistic regression analyses ($\alpha=0.05$).

Results: 221 surveys were received (response rate=29.8%). Respondents were most likely to treat: other ethnic groups (99%), patients with limited English proficiency (93%), LGBTQ+ adults (91%), HIV+ patients (88%), medically complex patients (87%), and children < 3 years old (83%). Respondents provided the least amount of treatment to homebound (15%), Medicaid (25%) and incarcerated (27%) patients. Respondents reported feeling very comfortable or comfortable treating: other ethnic groups (99%), LGBTQ+ adults (98%), Medicaid patients (95%), HIV+ patients (94%), and patients with limited English proficiency (92%). They were least comfortable treating homebound (53%) and incarcerated patients (71%). Low reimbursement rates (65%), not having portable dental equipment (59%), patients not showing up for appointments (43%), and paperwork being too burdensome (41%) were cited as the most important reasons for not treating underserved populations. Respondents were most interested in CE courses pertaining to treating medically complex (76%), mentally compromised (64%) and frail elderly (56%) patients.

Conclusion: Populations that alumni treated the most and least were those whom they felt the most and least comfortable treating, respectively, with the exception of treating patients with Medicaid. In spite of feeling comfortable treating this population, many respondents limit the number of patients with Medicaid they treat.

Supported by: Delta Dental of Iowa Foundation.

13. Demographic Trends in Third Molar Extractions



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Objective: Third molar extractions are a routine procedure performed by dentists and oral surgeons. The American Academy of Oral and Maxillofacial Surgery recommends that unerupted third molars be evaluated for extraction to prevent future complications, while multiple systematic literature reviews reported that there was not sufficient evidence to support clinical benefit from prophylactic extraction of third molars over no treatment. Therefore, we aimed to observe the demographic trends associated with the different types of extraction procedure codes.

Methods: Patient data were retrieved by searching Axium for all third molar extraction procedures in the Department of Oral & Maxillofacial Surgery (2008-2023). The search yielded a dataset of 21,161 patients and 69,171 teeth extracted. For each tooth extracted, the tooth number, associated chart number, age, gender, procedure code, and treatment date were reported. Using 6 types of extraction codes commonly associated with third molar extractions (D7140, D7210, D7220, D7230, D7240, D7241), we were able to relate patient variables and clinical presentation (fully erupted, soft tissue impacted, etc.) with age, gender, and arch location (maxillary vs. mandibular).

Results: The most common procedure code completed in association with a third molar extraction was D7240 (completely bony impaction). Mandibular third molars were most frequently completely bony impacted while the maxillary third molars were most likely to be fully erupted upon extraction. The extraction code varied with respect to patient age, with complete bony extractions occurring on average 8 years earlier than fully erupted third molar extractions.

Conclusion: There are differences in the trends of clinical presentations of maxillary and mandibular third molars at the time of extractions. Overall, mandibular third molars had a higher impaction rate (of any type of impaction) compared to maxillary third molars.

14. Patient Variable Effects on Pain Reports of Mandibular Molar Endodontics



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Objective: This study aimed to explore associations between buccal cortical plate thickness in proximity of mandibular molars undergoing endodontic treatment with two outcomes: (1) post-treatment surveyed pain reports and (2) anesthesia approaches necessary to facilitate primary root canal treatment of mandibular molars.

Methods: Subjects included individuals undergoing RCT by resident providers of a permanent first or second mandibular molar at the University of Iowa Endodontics Clinic. Pulpal and periapical diagnoses were established and a cone beam computed tomography (CBCT) scan was acquired. Local anesthesia (LA) was administered using a standardized protocol, and the level required was recorded. Each subject completed a six-question survey immediately after their appointment. CBCT measurements included the distance from the cortical plate to root apices (M2) and buccal cortical plate thickness (M3). Statistical analysis described results using logistic regression ($\alpha=0.05$).

Results: Fifty-five subjects were included in the study. Pulpal diagnosis was symptomatic irreversible pulpitis (SIP) and pulpal necrosis for 19 (35%) and 33 (60%) subjects, respectively. Level one anesthesia was sufficient for most subjects (n=45, 85%). Forty-seven subjects (n=54, 87%) reported no pain/non-painful sensations during administration of LA and 47 subjects (n=51, 92%) reported adequate anesthesia throughout the procedure. The odds ratios for a patient needing level two anesthesia or higher were 0.73 (95% CI: [0.43-1.09], p = 0.2) and 0.52 (95% CI: [0.15-1.69], p = 0.3) for M2 and M3, respectively.

Conclusion: Administration of two carpules of 2% lidocaine with 1:100,000 epinephrine via inferior alveolar nerve block followed by supplemental buccal infiltration with one carpule of 4% articaine with 1:100,000 epinephrine was effective in achieving profound anesthesia for most mandibular molars with SIP or pulpal necrosis. Within the limits of this study, there was no association between either distance from the cortical plate to root apices or cortical plate thickness and anesthetic efficacy.

15. A Novel Method for Evaluating Forces Present During RME Treatment



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Objective: Maxillary constriction is a common condition, often resulting in maxillary crowding, functional shifts, asymmetric jaw growth and posterior crossbites. The standard of orthodontic care is treatment with a rapid maxillary expander (RME) with the goal of separating the midpalatal suture. Few studies have attempted to measure the forces applied by the RME during treatment. The objective of this study is to pilot a novel method for measuring these forces.

Methods: Twenty-nine patients from 7 to 16 years of age undergoing rapid maxillary expansion with 2 or 4-banded Hyrax-style RMEs were recruited. Each expander underwent pre-clinical calibration before patient delivery. Patients returned daily for 2 jackscrew turns over 12-15 days. The force of each turn was recorded. Photos were captured and diastema width was measured at each visit. Intraoral scans were captured at each visit, and maxillary occlusal radiographs were taken after the final day of turning. The forces of the maxilla resisting expansion were estimated based on turn forces using the calibration curve created for each expander.

Results: Preliminary analysis corroborates previous findings that forces range from 0 to 20 lbs, with no decrease in force levels following sutural separation, indicating that resistance to expansion is not due to the midpalatal suture alone. Older patients (>12.5 y.o.) exhibited higher overall expansion loads compared with 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 after sutural separation. Older patients showed a higher skeletal width increase in 4-banded hyrax expanders compared to 2-banded ones.

Conclusion: Older patients presented higher overall forces and greater initial rates of load increase. Four-banded expanders resulted in more skeletal expansion. Statistical analysis of the outcomes is in progress.

16. Impact of Rapid Maxillary Expansion in the Periodontium



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Objective: Transverse maxillary deficiency is a common condition resulting in occlusal discrepancies among non-syndromic and syndromic patients. Scarce evidence is available regarding the occurrence and prevalence of hard/soft tissue deficiencies among patients undergoing surgically assisted rapid maxillary expansion (SARME) as compared to non-surgical rapid maxillary expansion (NSRME) approaches. The purpose of this study was to evaluate the effect of NSRME and SARME upon the periodontal structures.

Methods: A literature search was performed to identify studies that fulfilled pre-established eligibility criteria evaluating changes in the periodontium (e.g., probing depths) and hard/

soft tissue deficiencies (e.g., changes in alveolar bone, gingival recession, among others) within patients undergoing NSRME and SARME procedures.

Results: A total of 21 articles were included in the present review. Four of them evaluated the outcomes of both NSRME and SARME procedures, while 6 and 11 studies analyzed NSRME alone and SARME alone, respectively. No quantitative analysis was possible due to the heterogeneity of the extracted data. The incidence of hard (e.g., changes in buccal bone width/height) and soft tissue deficiencies (e.g., gingival recession, keratinized tissue, clinical attachment level) is slightly increased among patients undergoing maxillary expansion with worsened outcomes during NSRME procedures.

Conclusion: The impact of NSRME and SARME upon the periodontium remains inconclusive. However, the qualitative analysis suggested that the onset of hard and soft tissue deficiencies is relatively higher among patients undergoing NSRME procedures when compared to SARME.

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

Oral Session 4

17. Tobacco and Nicotine Effects on Microbiome-Immune-Metabolome Axis



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Objective: 22.0% of Americans 12 and older report using tobacco products or vaping nicotine in the past 30 days, and 8.5% of the population report nicotine dependence. While the impacts of these habits on the subgingival microbiome are studied, the impacts of tobacco and nicotine use on the total oral environment and the mechanisms behind metabolic and immune changes, remains unknown.

Methods: Saliva, mucosal biopsies, and swabs were collected from 33 periodontally healthy cigarette smokers, e-cig users, and controls. Habit duration and frequency were recorded. Bacterial DNA was isolated, amplified, and sequenced on the Illumina Miseq 2x300bpPE platform. Multiplex assays quantified adipokines and cytokines; significance was determined by Tukey HSD. Metabolite peaks from GC-MS were annotated against the Small Molecule Pathway Database, with significance assessed through enrichment analysis. Salivary cotinine levels were measured.

Results: Marked differences were observed in the microbial composition, validating previous oral microbiome studies. The saliva of cigarette smokers exhibited elevated levels of IL-5; the pro-inflammatory cytokine identified in higher levels of bronchial lavage in smokers. Histamine, an associated metabolite, mirrored the pattern. Amino adipic acid, a metabolite associated with cardiometabolic diseases, and

mannose, an insulin-sensitivity metabolite, were significantly elevated in cigarette smokers. Interestingly, hypotaurine, a metabolite with anti-oxidant properties and inflammatory regulatory roles, was increased in cigarette smokers followed by e-cig users. Multiomics analysis reveals an IL-10 predominant network in controls, compared to IL-17, IL-1, and TNF-alpha predominant networks in e-cig users and cigarette smokers, even in the absence of any clinical oral disease.

Conclusion: Our study delineates tobacco and nicotine-specific microbial, immune, and metabolic alterations, presenting potential biomarkers and contributing valuable insights into the intricate dynamics of the oral environment.

18. Changes in the Salivary Microbiome During Fixed Orthodontic Therapy



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Objective: Patients undergo fixed orthodontic treatment (FOT) to improve dental and facial aesthetics, correct occlusal function, and/or eliminate traumatic occlusion. However, many patients, especially teenagers, struggle to maintain good oral hygiene. This can result in plaque-related complications such as periodontal diseases, caries, and white spot lesions. Plaque-mediated diseases result from imbalances in the microbiome leading to an enrichment of disease associated pathogens in

the microbial community. Saliva samples provide information about microbes, immune signatures, and metabolites present in the oral environment and are easy to collect during routine visits. We aimed to comprehensively characterize how the microbial composition of the salivary environment changes throughout FOT.

Methods: This cross-sectional study analyzed saliva samples from 55 orthodontic patients, aged 11-20 years, and 14 periodontally healthy control subjects not undergoing orthodontic treatment, matched for age, gender, and dentition. Unstimulated saliva samples were collected at 6 months, 12 months, or 24 months into orthodontic treatment. DNA was isolated from saliva samples, quantified, and normalized. The V3-V4 regions of the 16S rRNA gene were amplified and sequencing was completed using the Illumina MiSeq platform to collect 300 base pair paired-end reads. Sequences were annotated against the SILVA database.

Results: FOT was associated with a significant reduction in bacterial diversity compared to controls; however, the diversity was similar among different time points. Timepoint-specific shifts were observed in the salivary microbiome of FOT cohorts. Later time points (12 and 24 months) showed increased levels of several health-associated species, such as Eikenella and Bifidobacterium, and a reduction in disease-associated species, such as Sacccharibacteria, Selenomonas, Treponema, and Fusobacterium.

Conclusion: Our cross-sectional analysis of the salivary microbiota is a step towards comprehensively understanding how the salivary microbiome changes throughout FOT. In the future, this foundation may aid in patient risk profiling and disease intervention during orthodontic treatment.

19. Exploring the DNA and RNA Virome of the Oral Cavity



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Objective: The oral microbiome plays a critical role in the prevention, development, and progression of many oral disease, including periodontitis, caries, and oral cancer. An important part of our ongoing investigation into the dynamics of oral health and disease is the potential clinical significance of the viral component of the oral microenvironment, which is still largely unexplored. As a start to addressing this, we conducted a secondary data analysis to determine how the composition and function of the oral virome may influence oral health conditions.

Methods: Metagenomic/metatranscriptomic data from Belstrøm et al. (PMC5624903) was downloaded through the NCBI SRA. 30 participants in three groups: periodontitis, caries, and health were recruited and stimulated saliva collected. BBDuk and Bowtie2 performed quality/adaptor and human filtering with MEGAHIT and SPAdes for assembly. VirSorter2, DeepVirFinder, VIBRANT, and geNomad were applied to identify viral contigs, and final dereplication and quality checking with CheckV. DRAM-v and iPhoP were used for genome annotation and analysis. Microbial genomes (MAGs) were recovered using

MetaWRAP and analyzed with DRAM and GTDB-tk.

Results: Overall we recovered a total of 4536 deduplicated DNA viral genomes and 795 RNA viral genomes, ranging in length from 5kb-382kb and 1kb-21kb respectively. The top predicted microbial hosts were *Streptococcus*, *Prevotella*, *Fusobacterium*, *Capnocytophaga*, and *Actinomyces*. Functionally, we found in the DNA viruses a wide range of potential auxiliary metabolic genes involved in carbon and nitrogen utilization and in the RNA viruses primarily ribonucleotide reductase genes. Finally, we recovered a total of 1014 MAGs with an average of 34 recovered per sample.

Conclusion: Here we have conducted a comprehensive analysis of the DNA and, for the first time, the RNA oral virome in conjunction with assembly-based recovery of microbial genomes, adding to our understanding of the complete oral microbiome in relation to health and disease.

Supported by: NIDCR T90 DE023520.

20. Multi-Omics Comparison of the Oral Mycobiome



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Objective: The human oral microbiome, a vital component of oral and systemic diseases constitutes a variegated horde of microbes. However, the microbial population other than

bacteria has been understudied. The oral mycobiome has gained recent attention but was analyzed specifically for *Candida albicans* in most studies. A comprehensive analysis of the organisms over various multi-omics data is lacking.

Methods: In this direction, we developed a pipeline to preprocess multi-omics datasets and map them against standard fungal databases. We retrieved the raw sequences of metagenomics, metatranscriptomics, and metaproteomics from studies by Belstrøm et al. published in 2016 and 2017. Each omics dataset encompassed data from 10 healthy individuals and 20 patients (10 with dental caries and 10 with periodontitis). Metagenomics and metatranscriptomics sequences were processed and mapped with Kraken2 and HUMAnN3. Metaproteomics data was preprocessed with MaxQuant and analyzed via UniProt/SwissProt. Specialized codes for downstream analysis were created using RStudio.

Results: The organismal and functional abundance across the three multi-omics data were analyzed. Species-level mapping was available for 92 species in metagenomics and metatranscriptomics, while metaproteomics yielded 40 species. On the genus level, *Candida*, *Saccharomyces*, *Schizosaccharomyces*, and *Aspergillus* were common among all the multi-omics data. Functionally, replication-associated proteins like hta1, act1, fal1, hta2 and eft1 were identified. Aligning to this, the pathway mapping revealed mitotic checkpoints, cytoplasmic translation, and protein folding. While no discernible patterns link the diseases across these levels, certain species, genes, and pathways were notably more prominent than others.

Conclusion: Our cutting-edge research involves a comprehensive analysis of taxonomic and functional data across the three types of omics types from the oral microbiome. By utilizing our integrated methodology, we strive to identify biomarkers, distinguish between virulent organisms and commensals, and explore host-gene interactions. This refined comprehension will lay the foundation for more accurate and personalized oral healthcare.

21. Machine Learning Approach Identifies Dysbiotic Oral Communities in Multiple Sclerosis



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Objective: Multiple Sclerosis (MS), an inflammatory demyelinating disease of the CNS, affects roughly 2.3 million people worldwide, with Relapsing-Remitting Multiple Sclerosis (RRMS) making up the majority of MS cases. Although the precise pathobiology of RRMS is unknown, the gut microbiome has emerged as a potential environmental factor. However, the importance of the oral microbiome (the next most diverse microbiome) as a potential environmental factor in RRMS is unknown. Prior studies revealed oral microbial dysbiosis in non-oral diseases, including Alzheimer's, Atherosclerosis, and rheumatoid arthritis.

Methods: To determine the importance of oral microbiome in RRMS, we collected saliva from RRMS (n=50) and healthy controls (HC, n=50). Microbial taxonomy was determined by shotgun metagenomic sequencing followed by reads processing and taxonomy assignment through the metawrap pipeline. Along with microbial diversity and abundance analysis, we performed topic modeling, a machine learning approach, to assess latent interactions occurring among microbes, thus providing a representation of the community of bacteria associated with RRMS or HC.

Results: RRMS patients showed oral dysbiosis characterized by reduced alpha diversity compared to HC. Beta diversity showed distinct clustering between the two groups when utilizing the compositional and absolute abundance approaches, indicating a significantly different microbiome in RRMS. Univariate analysis revealed an enrichment of one of the red complex species, *Porphyromonas gingivalis*, in RRMS and a depletion of several *Streptococcus* and *Actinomyces* genera. Using topic modeling, we identified several microbial communities assigned to either HC or RRMS.

Conclusion: This is the first oral microbiome study on a large cohort of RRMS patients using shotgun metagenomics that utilizes compositional and machine learning approaches to identify specific bacteria and dysbiotic communities linked to RRMS. Our study brings us closer to harnessing the potential of microbial-based therapies for treating RRMS.

Supported by: NIH F31 DE033564; NIDCR T90 DE023520.

22. Standardizing Protocol for Obtaining Collagenase from Streptococcus Mutans



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Objective: This study aimed to standardize a protocol to obtain collagenase from *Streptococcus mutans* (UA159), evaluating its presence and activity.

Methods: *S. mutans* cells were grown at 37°C and 5% CO₂ for 48 hours in 1) THYE with 50mM MOPS, pH 7.2 (T); or 2) CDM with 0.2% glucose + 0.5% peptone + 0.2% sodium bicarbonate, pH 7.2 (C). Following incubation, the cells were separated from the supernatant by centrifugation. The pH of supernatants was adjusted to 7.2, filter sterilized, dialyzed, and concentrated. The cell pellets were washed and re-suspended in PBS. The cells were disrupted with glass beads (1, 3 and 5min), and centrifuged for 5min (16,000xg) at 4°C to separate cell debris from the intracellular content. Total protein was quantified via BCA assay. SDS gel electrophoresis and gelatin zymography assayed the protein profile and gelatinolytic activity of the supernatant and intracellular content.

Results: The supernatants from C and T protocols presented 4.2mg/ml and 2.8mg/ml of total protein, respectively. The intracellular content of cells cultured in CDM showed higher protein concentration, with higher amounts from longer cell disruption times (0.23mg/ml). The intracellular content from protocol T presented 0.0042mg/ml of total protein. Supernatant from the C protocol showed a greater number of bands ranging from ~28 to ~180KDa while protocol T showed fewer bands. All groups of intracellular content showed different band patterns ranging from ~28 to ~150KDa. Proteins obtained with the C protocol showed a more diverse protein profile in comparison to the T protocol. Zymography gels showed gelatinolytic activity for supernatant from the T protocol.

Conclusion: Thus, the protocol using THYE media was most suitable for obtaining active enzymes from *S. mutans*. Additional experiments will clarify the proteins' potential to degrade collagen and dentin matrix.

Supported by: NIDCR R90 DE024296.

Oral Session 5

23. Analysis of Probiotic Candidates' Suppression of Candida albicans Mediated Inflammation



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Objective: *Candida albicans* has been linked to oral diseases ranging from dental caries to oral cancer. It appears to enhance the cariogenicity of the dental pathogen *Streptococcus mutans* and is also associated with pro-inflammatory changes in the oral microbiota. The goal of this project was to test probiotic candidates, previously isolated from children with a caries free history and partially screened for properties related to caries prevention, for inhibition of *C. albicans* and suppression of inflammation.

Methods: *C. albicans* was grown in the presence of probiotic candidates in addition to health and disease associated controls to evaluate inhibition of *C. albicans* and to determine their influence, or lack of, on hyphae formation. Promising candidates were assayed using quantitative polymerase chain reaction (QPCR) to quantify relative expression of potential oncogenesis related inflammatory cytokines, transcription factors/genes and enzymes in co-cultures of probiotic candidates with *C. albicans* in two squamous cell carcinoma cell lines and epithelial cell models.

Results: Candidates screened would ideally have the ability to coexist with *C. albicans* without inducing hyphae formation and limit cellular inflammatory response to *C. albicans*. None of the candidate strains tested had the ability to inhibit the growth of *C. albicans*; however, four of the nine candidates displayed the ability to reduce hyphae formation or coexist with *Candida* without inducing increased hyphae formation indicating minimal or reduced stress response from *C. albicans*. QPCR revealed two candidates, A2 and A3, significantly decreased expression of BCL3, BIRC3, JUN, IFIT3, SERPINE1, MMP-1, IL-6 and IL-8 in cells co-cultured with *C. albicans* compared to cultures of *C. albicans* alone in the three cell cultures.

Conclusion: Certain bacterial strains from children with a caries free dental health history have the potential to suppress chronic inflammation which may aid in the prevention of oral disease associated with *C. albicans*.

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

24. Orchestrating Oral Chronic Graft-Versus-Host Disease: The Tryptophan Connection



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Objective: Graft-versus-host disease impacts the oral mucosa in >50% of allogeneic stem cell transplant patients. The immune-mechanism at this interface site is poorly defined. While evidence supports a critical role of end-stage small molecules—metabolites—in intestinal GVHD, the oral environment lacks such investigations. The present study analyzed the longitudinal shifts in the metabolites and key immune mediators in HSCT patients.

Methods: Saliva and oral tissue biopsies from 14 patients who underwent matched-unrelated-donor allogeneic hematopoietic stem cell transplantation HSCT (trial:NCT02356159) were collected pre-transplant, 6-months, and 1-year post-transplant, and at oral cGVHD onset. Longitudinal shifts in 120 metabolites were determined using gas chromatography/mass spectrometry (GC/MS). 255 critical immunoregulatory genes were analyzed using a targeted gene expression analysis (nCounter Human Inflammation v2 Panel), followed by appropriate statistical tests and correlation analyses.

Results: Of the 14 patients, 10 developed cGVHD and 4 were asymptomatic at one-year follow-up. Pro-inflammatory genes, including HLA-DRA, HLA-DRB1, CD86, TNF, FASLG, IRF1, IRF3, IRF7, and IFN-γ, were significantly increased, and a common immediate-early gene, JUN, was decreased at the onset of cGVHD. The kynurenine:tryptophan ratio decreased across the time-points in the cGVHD group, while the control group exhibited the opposite trend. In addition, several pro-inflammatory metabolites including phenylalanine, methionine, valine, and threonine were increased in the cGVHD cohort, while anti-inflammatory and homeostatic metabolites including adenosine, alpha-ketoglutarate, phosphoenolpyruvate, and N-acetyl glutamine were decreased. Interestingly, tryptophan and indolelactic acid strongly correlated with critical immunoregulator IRF7 in the cGVHD-onset lesions, underscoring the potential role of these metabolites in cGVHD.

Conclusion: Our results from this prospective trial demonstrate the critical immune-regulatory role of Tryptophan:Kynurenine pathway in the onset of oral mucosal cGVHD. Larger studies are needed to validate these associations and further define the microbiome contribution to this immune-metabolite axis.

Supported by: American Cancer Society Seed Grant IRG -15-176-41; Intramural Research Program 1ZIADE000747-07.

25. Transcriptome of Irradiated Rat Mandibles and Implant-Infiltrating Cells Within Defects



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Objective: Radiotherapy is an effective adjunctive treatment for tumor resection, improving the treatment outcome. However, it can cause impaired and delayed bone healing by upregulated inflammation and increased oxidative stress. MicroRNAs exert epigenetic control over gene expressions and have been shown to modulate various physiological and pathological processes. The objective of the current study is to explore transcriptional changes in irradiated mandibles, including mRNAs and microRNAs, to identify potential therapeutic targets for improving bone healing.

Methods: Rats received 35 Gy radiation in 5 fractions to the mandible. Mandibles were harvested one day after treatment, and bulk mRNA and microRNA sequencing were conducted. In a separate experiment, a 4 mm critical-sized defect was created in rat mandibles, implanted with a collagen sponge, and subjected to the same radiation regimen two weeks post-surgery. Three days after the final dose, collagen implants were harvested, and sequencing was performed. Differentially expressed genes and enriched gene sets related to osteogenesis, osteoclastogenesis, inflammatory, and oxidative stress pathways were identified. Selected gene and microRNA expression changes were validated using qRT-PCR.

Results: Sixteen genes and one microRNA were differentially expressed in the irradiated mandibular bone. Radiotherapy enriched gene ontology terms associated with osteoclastogenesis, inflammation, and oxidative stress. Additionally, osteogenesis and vascularization processes were enriched in the control. In the collagen implant, 135 genes and 24 microRNAs were differentially expressed. Infiltrating cells exhibited enrichment in inflammatory and wound-healing processes. *Alp*, *Ocn*, and *Runx2* were downregulated in the irradiated mandible, while *Csf1* and *Noggin* were upregulated. Overall, osteoinductive genes and microRNAs tended to decrease post-irradiation, while anti-osteogenic and osteoclastogenic genes and microRNAs increased post-radiotherapy.

Conclusion: Bone tissue and implant-infiltrating cells' responses to ionizing radiation are regulated at the transcriptional level, including post-transcriptional regulation by microRNAs. Notably, inflammation and bone resorption processes are increased, accompanied by reduced bone formation processes.

Supported by: University of Iowa College of Dentistry P3; NIH R01 DE026433; The James S. and Janice I. Wefel Memorial Research Award.

26. Assessing the Role of Methyltransferase SET7 in Lipopolysaccharides-Induced Periodontal Inflammation



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Objective: Histone methylation on histone 3 lysine 4 (H3K4) is generally considered an active regulatory event for the inflammatory gene expression. This study aimed to investigate the role of histone methyltransferase SET7 in the NF-κB-related pro-inflammatory cytokines gene expression triggered by the *Porphyromonas gingivalis* (*P. gingivalis*)-derived lipopolysaccharides (LPS).

Methods: Primary human periodontal ligament cells (hPDLs), human telomerase immortalized gingival keratinocytes (TIGKs) and human gingival fibroblasts (HGFs) were challenged by *E. coli* LPS (5μg/ml) and *P. gingivalis* lipopolysaccharide (1μg/ml) respectively, with or without the pretreatment of a SET7 specific inhibitor, (R)-PFI-2. RNA interference of SET7 was applied to hPDLs, which were also challenge by *P. gingivalis* LPS. The expression of NF-κB subunit p65, phospho-p65 and SET7 in hPDLs was measured by immunoblotting. Gene expression of *IL8*, *IL6*, *ICAM1*, *MCP1(CCL2)*, and *TNFA* in TIGKs and HGFs was measured by real-time qPCR. In an experimentally induced periodontitis model in rat, the expression of p65, phospho-p65 and SET7 in the gingival tissue was detected by real-time qPCR and immunoblotting.

Results: The *P. gingivalis* LPS induced p65, phospho-p65 and SET7 upregulation in hPDLs. SET7 inhibitor (R)-PFI-2 and RNA interference of SET7 significantly suppressed the NF-κB p65 activation. The transcriptional expression of *IL8*, *IL6*, and *ICAM1* increased in TIGKs challenged by *E. coli* LPS. The expression of *IL8*, *IL6*, *MCP1(CCL2)*, and *TNFA* increased in HGFs challenged by *P. gingivalis* LPS. The upregulation of *IL8* and *ICAM1* in TIGKs, and *IL8*, *IL6*, *MCP1(CCL2)*, and *TNFA* in HGFs were both inhibited by the SET7 inhibitor (R)-PFI-2. The expression of NF-κB and SET7 both increased in the gingival tissue in the rat periodontitis model.

Conclusion: These results demonstrate that SET7 expression increases during inflammation triggered by lipopolysaccharides and in the animal periodontitis model. Inhibition of the SET7 catalytic activity attenuates pro-inflammatory gene expression in periodontal resident cells, indicating that histone epigenetic modifications catalyzed by SET7 are involved in the pathology of periodontal disease.

27. Novel Fatty Acid Treatment for Periodontitis With Chronic Kidney Disease



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Objective: Chronic kidney disease (CKD) characterized by progressive kidney function decline, affects over 10% of the population, and nearly 1.1 million deaths are due to CKD annually worldwide. Chronic inflammation is a common pathway for CKD development and progression and can trigger and exacerbate CKD. Periodontitis (PE) is a leading inflammatory oral disease affecting nearly half of U.S. adults. Accumulating evidence has demonstrated a strong correlation between PE and CKD. Medium-chain fatty acids (MCFAs) have been reported to reduce inflammation by regulating inflammation and immune-related pathways and reducing oxidative stress. This study investigates the therapeutic potential of 6-hydroxyhexanoic acid (6-HHA), an omega-hydroxy MCFA, for treating PE and improving kidney function and overall health outcomes in individuals with CKD.

Methods: To test the inhibitory function of 6-HHA, human monocyte-derived macrophages and human gingival fibroblasts were challenged with lipopolysaccharide and co-treated with 6-HHA at different concentrations. Lipopolysaccharide was injected twice a week into the interdental region between maxillary molars of mice to induce periodontal inflammation, and then 6-HHA was administered twice a week. Human proximal tubule epithelial cells were challenged with human recombinant IL-1β protein and treated with 6-HHA at different concentrations. The transcript and protein levels of proinflammatory cytokines were measured by qPCR and ELISA.

Results: Our studies have identified that 6-HHA strongly inhibits the proliferation of periodontal pathogenic bacteria and reduces inflammation in PE-associated human cells and mouse models. We also found that 6-HHA has the potential to downregulate proinflammatory cytokines in human kidney cells. This evidence suggests that 6-HHA has strong potential as a therapeutic tool for PE patients with CKD by mitigating inflammation locally and systemically.

Conclusion: 6-HHA can effectively reduce inflammation in periodontitis and chronic kidney disease and slow the progression of chronic kidney disease. It can be developed into a novel therapeutic tool for treating periodontitis and improving kidney function.

Supported by: NIH R01 DE026433; NIDCR T90 DE023520.

28. Type I Interferon Plays Protective Role in Murine Ligature-Induced Periodontitis



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Objective: We previously found that, interferon-β, a classical type I interferon (IFN) member, was significantly associated with severe periodontitis and severe tooth loss clinical phenotypes. Therefore, we aim to investigate the role of IFN-β in a murine ligature-induced periodontitis (LIP) model

Methods: The alveolar bone loss measured from microCT in global *Ifnar1*^{-/-} was compared to that in wild-type (WT) mice in the LIP model. We prepared live single immune cell (CD45⁺) suspension in ligated gingiva from both WT and *Ifnar1*^{-/-} mice after flow sorting, then performed single-cell RNA sequencing through 10X Genomics. Additionally, the bone loss was evaluated between the *Ifnar1*fl/fl/*LysM*Cre (conditional knockout in myeloid cells) and the littermate control *Ifnar1*fl/fl/*LysM*wt- mice. We compared the osteoclast numbers between the *Ifnar1*fl/fl/*LysM*Cre and the *Ifnar1*fl/fl/*LysM*wt- mice in the LIP model. We also compared the transcriptional expression of immune genes from the conditional knockout to the control mice

Results: Global *Ifnar1*^{-/-} mice exhibited significantly more bone loss than WT mice (p=0.037). Myeloid cells, especially neutrophils, were elevated in the *Ifnar1*^{-/-} mouse gingiva in comparison to the WT samples. *Ifnar1*fl/fl/*LysM*Cre mice manifested significantly more alveolar bone loss than the *Ifnar1*fl/fl/*LysM*wt- mice (p=0.005). More osteoclasts were present in the *Ifnar1*fl/fl/*LysM*Cre mice than that in the control mice. The transcription of *Il17*, *Csf3r*, *Il18rap*, *C5ar1*, and *Il1b* was significantly upregulated in the *Ifnar1*fl/fl/*LysM*Cre mice when compared to the *Ifnar1*fl/fl/*LysM*wt-mice (p<0.05)

Conclusion: The myeloid lineage-specific type I interferon response dampens the periodontal inflammation and alveolar bone loss in the ligature-induced periodontitis model

Supported by: NIH R01 DE032307.

29. Assessment of Completeness and Laterality of Cleft Lip Presentations



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Objective: Clefts of the lip may or may not involve the complete vertical thickness of the lip resulting in complete or incomplete phenotypes, respectively. This study evaluates if there is a side preference for completeness on non-syndromic cases with asymmetric bilateral and unilateral clefts of the lip, irrespective of cleft palate (NSCL/P).

Methods: Cleft laterality and completion data were evaluated from 3 multiethnic cohorts from North and South America, Asia, and Africa including 1,517 individuals with NSCL/P. Of these, 56 and 1,461 cases had asymmetric bilateral or unilateral CL/P respectively. Cleft completeness per side and its relationship with sex, ethnicity, and race were compared via Chi-square test or Fisher's exact test at a significance level of 0.05 using SAS® System v9.4, SAS Institute Inc. in Cary, NC, USA.

Results: Amongst asymmetric bilateral cases, left side completion was significantly more frequent than the right side (76.8% vs. 23.2%; $p < 0.001$). Hispanics more often exhibited right completeness compared to non-Hispanics (46.2% vs. 16.3%; $p = 0.025$). No associations were found for sex or race. For unilateral cases, completeness was not associated with a specific side (75.5% for left side vs. 78.8% for right side; $p = 0.152$). Whites were less likely to exhibit completeness compared to Asians or other racial groups (68.8% vs. 84.9% or 81.5%; $p < 0.001$). Conversely, Hispanics more frequently showed completeness compared to non-Hispanics (85.8% vs. 71.2%; $p < 0.001$). No sex differences were observed.

Conclusion: In cases of NSCL/P with bilateral asymmetry, the left side is more often complete compared to the right side. While unilateral left cases occur more frequently, there was no association between completeness and side.

Supported by: NIDCR U01 DE024425, R21 DE016930, R01 DD000295, R37 DE008559, R01 DE011931, R00 DE024571, R01 DE016148; NIGMS U54 GM133807; CDC NCBDD R01 DD000295; University of Iowa College of Dentistry Student Research Program

30. Tale of Two Faces: TFAP2 in Midface Development and Dysplasia



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Objective: Neural crest cells (NCCs) form unique facial structures during embryonic development thanks to genetic programs specific to each region. Typifying this idea, loss of positional factors (e.g., midface-specific ALX) results in position-specific craniofacial disorders (e.g., frontonasal dysplasia, clefts in the forehead and nose). Despite this knowledge, how genes connect into discrete regulatory nodes—particularly the midface—remains poorly understood. We hypothesized that the TFAP2 transcription factors, known regulators of NCC development and facial morphology, function in the midface positional program.

Methods: We combined complex mouse genetics (CRE-loxP system, epistasis analyses), gene expression profiling (single-cell RNA-seq, real-time PCR), and epigenomics (ChIP-seq, ATAC-seq) to examine the phenotypic and molecular consequences of losing *Tfap2a* and *Tfap2b* in NCCs.

Results: Combined loss of *Tfap2a* and *Tfap2b* genes in NCCs resulted in a fully penetrant midfacial cleft compared to controls or loss of a single paralogue. Single-cell RNA-seq revealed that loss of *Tfap2* genes dysregulates numerous components in the midface positional program. These included decreased transcript levels for *Msx1*, *Pax3/7*, and most notably the frontonasal dysplasia-associated ALX transcription factor genes (*Alx1/3/4*). The latter is of interest because the *Tfap2*-associated midface cleft phenocopies those in *Alx* compound mutants and whose functions include NCC patterning and skeletal differentiation. Combined ChIP- and ATAC-seq profiling suggested that *Alx* gene expression is directly dependent on TFAP2 function. Finally, deploying epistasis analyses, we observed that reducing *Alx3* gene dosage in single *Tfap2* mutant backgrounds resulted in midfacial anomalies synergistically increasing in both penetrance and phenotypic severity.

Conclusion: Our findings identify TFAP2 transcription factors as key regulators of midfacial development and that the TFAP2-ALX regulatory node is a crucial component of its program. Ongoing studies aim to determine how signaling pathways known to regulate the midface axis integrate into our newly discovered program.

Supported by: NIDCR F31 DE032881, T90 DE023520, F32 DE029995, 2R01 DE012728, R01 DE029193, R00 DE026823; MICIN/AEI /10.13039/501100011033 (PID2020-117640RB-I00); University of Iowa Faculty Start Up Fund, University of Iowa College of Dentistry Seed Grant; University of Iowa Graduate and Professional Student Government (GPSG).

31. A Role for PRMT5 in Oral & Skin Epithelial Development



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Objective: During development, the initially single-layered epithelium of the presumptive skin or oral cavity requires precise coordination of cell proliferation and differentiation to successfully execute stratification. Defects in these events during development lead to congenital anomalies including harlequin ichthyosis or congenital granular epulis whereas accumulation of harmful mutations in adulthood can lead to surface or oral squamous cell carcinoma. Protein Arginine Methyl Transferase 5 (PRMT5)—an enzyme that catalyzes methylation of arginine residues in critical proteins, including histones and transcription factors—is upregulated in various carcinomas and correlates with poorer prognosis. While inhibition of PRMT5 has been shown to have anti-cancer properties, the mechanisms behind this effect are unknown. Interestingly, PRMT5 has been identified as necessary to maintain a progenitor, stem-cell fate in both germ-cell and limb development as well as a variety of cancers. Therefore, we hypothesize that PRMT5's methylation of histones and transcription factors drives a gene expression program that impedes differentiation allowing the maintenance of a stem-cell phenotype.

Methods: To test this hypothesis, we used conditional mouse genetics to delete *Prmt5* from the early (E7.5) ectoderm. These embryos were then subjected to phenotypic, histological, and molecular analysis.

Results: Consistent with a critical role for PRMT5 during this process, epithelial loss of *Prmt5* resulted in gross skin defects, reduced skin barrier function, and reduced postnatal viability. Histological analyses of control and mutant skin revealed severe defects in epidermal stratification, including major reduction of the proliferative basal layer. Molecularly, sc-RNA/ATAC-seq suggests that loss of PRMT5 leads to the emergence of an atypical keratinocyte-like cell population not found in control samples. Ongoing work aims to clarify this atypical cluster and how its presence may disrupt epidermal development.

Conclusion: Collectively, our findings have identified a critical role for PRMT5 in epidermal development and provide a novel model in which to dissect its molecular function in this process, providing insight into its role during tumor progression.

Supported by: NIDCR T90 DE023520; University of Iowa College of Dentistry P3.

32. MEMO1 is Required for Enamel Formation



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Objective: Mineralization of dental enamel is essential for tooth longevity. Amelogenesis imperfecta (AI) is a rare congenital disorder characterized by abnormal enamel development, resulting in discolored, pitted and malformed teeth. AI is estimated to impact 1 in 15,000 births per year in the United States. Previous works have heavily focused on the role of enamel matrix proteins and their role in the pathogenesis of AI. Currently, 50 percent of all cases have an unknown genetic etiology necessitating the study of novel proteins within the ameloblast—the enamel generating cells. We previously identified a role for the cytoskeletal regulating protein MEMO1 in craniofacial mineralization. Here, we extend these findings and report the role of MEMO1 in the context of enamel mineralization.

Methods: In mice, we conditionally deleted Memo1 from oral ectoderm derived ameloblasts and preformed uCT, SEM and molecular profiling of mutant and control animals.

Results: We found that compared to littermate controls, Memo1 knockout mice exhibited significant enamel mineralization defects like human AI. Furthermore, molecular analyses (sc-RNAseq, RT-PCR) suggested an increased number of early-stage ameloblasts and a reduced number of late-stage ameloblasts in mutants, relative to controls. GO term analysis of differentially expressed genes in control and mutant clusters identified a decrease in genes associated with “secreted”, “microtubule-based process” and “biomineralization” pathways—providing testable hypotheses for MEMO1 function in the ameloblast.

Conclusion: Overall, these findings provide a novel role for MEMO1 in the ameloblast. This knowledge has the potential to contribute to the improvement of risk assessment and diagnostic strategies for bone and enamel-related mineralization defects.

Supported by: NIH R00 DE026823, T90 DE023520; University of Iowa College of Dentistry Seed Grant.

33. Cross-Repression of *Pitx2* and *Tfap2a/b* Regulates Tooth Initiation Site



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Objective: Tooth development starts with formation of dental lamina (DL), which determines the future dentition localization. How the dorsal-ventral axis of vertebrate jaw is established, particularly the determination of DL position, remains as a critical and unresolved question.

Methods: To identify potential genes and transcriptional regulatory networks that control specification of DL within the continuous naive mandibular epithelium, we utilized single-cell Multiome-seq and Laser Microdissection coupled low-input RNA-seq to profile gene expression and open chromatin of mandibular epithelium during tooth initiation. Additionally, *Pitx2* general knockout (*Pitx2* KOs) and *Tfap2a/Tfap2b* ectodermal double knockout (*Tfap2a/b* EDKO) mouse models were employed to determine roles of these domain specific transcription factors.

Results: We comprehensively identified TFs and signaling pathways that are differentially expressed along dorsal-ventral axis of mandibular epithelium. With two knockout mouse models: *Pitx2* KOs (dental lamina specific TF) and *Tfap2a; Tfap2b* EDKO (ventral surface specific TFs), we found *Tfap2a* and *Tfap2b* establish the ventral epithelium identity by promoting expression of multiple WNT ligands, which active WNT pathway activity in ventral mesenchyme. Similarly, *Pitx2* promotes expression of FGF ligands in DL. In addition, we found cross-repression of different domain specific TFs (including *Pitx2* and *Tfap2a/b*) with both *in vitro* and *in vivo* systems. Moreover, we found association of mesenchymal SHH and WNT pathway activity, regulated by epithelium domain specific TFs including *Pitx2* and *Tfap2a/b*, at the tooth initiation site.

Conclusion: These results uncover a previously unknown molecular mechanism involving cross-repression of domain specific TFs including *Pitx2* and *Tfap2a/b* in patterning the dorsal-ventral axis of the mouse mandible, specifically the regulation of tooth initiation site.

Supported by: NIDCR R21 DE029828, R01 DE033009.

biomimetic gelatin scaffold for hADSCs and their influence on cell proliferation, differentiation and secretome.

Methods: 3D gelatin nanofibrous (GF) scaffolds were made by thermal induced phase separation & porogen leaching. The hADSC was cultured in 2D, and 3D (hanging drop and GF) conditions. Conditioned medium (CM) from three culture methods were collected and cultured with hADSC. The influence of CM for cell proliferation and osteogenic differentiation was tested by MTS, ALP activity and AR S Red staining, respectively. ELISA testing was used for cell secretome analysis.

Results: The conditioned medium from the hanging drop method promotes cells proliferation and osteogenic differentiation, and hADSC secretes more reparative growth factors in a 3D culture condition compared to 2D culture.

Conclusion: The hanging-drop 3D cultures of hADSC outperformed the 2D traditional cultures along with the 3D nanoscaffold culturing when comparing cell proliferation, osteogenic differentiation and secretome.

Supported by: NIDCR R03 DE027491, R01 DE029159, T90 DE023520; The Department of Oral and Maxillofacial Surgery.

36. Pilot Testing of RNA Based Therapeutic Approaches for Bone Regeneration



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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 without negative side effects.

Methods: Human BMP2 and BMP7 genes were cloned into a circular RNA system that was recently developed to enhance protein production. The circular RNA encoding EGFP and BMP2 were transfected into 293T cells and were used to measure *in vitro* stability and translation. GFP protein production was visually inspected using fluorescent microscopy and qPCR was used to assess BMP2 circular RNA stability *in vitro*.

Results: Fluorescence measurement of EGFP showed there were more cells expressing EGFP, the cells had brighter fluorescence on Day 2 compared to Day 1, and strong EGFP fluorescence was observed until 1 week. The preliminary qPCR results showed minimal degradation of BMP2 RNA from Day 1 to Day 2—only a 17% decrease in BMP2 RNA expression. However, we saw a quick decrease in BMP2 mRNA from Day 4.

Conclusion: This study successfully cloned human BMP2 and BMP7 into circular RNA production plasmids and showed that circular RNA could be efficiently translated *in vitro*. This preliminary testing of stability and translation of circular BMP2 *in vitro* shows that BMP as circular RNA has higher stability and translation efficiency than linear RNA. The cloned BMP2 and BMP7 will be useful in future studies to further investigate

RNA expression through additional qPCR testing and ELISA testing. Additional optimization of the circular RNA system 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 R21 DE029828, R01 DE033009.

37. Novel Metabolite-Derived Microparticles for Intracellular Drug Delivery and Bone Regeneration



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Objective: Alpha-ketoglutarate (AKG), a key Tricarboxylic acid cycle component, has garnered attention for its antiaging properties in various organisms and ability to mitigate bone loss and enhance bone anabolism in

mice. Nevertheless, AKG's utility is hindered by its limited cell permeability, high dosage and long-term systemic administration requirements. Dimethyl alpha-ketoglutarate (DMAKG) is a cell-permeable derivative of AKG and can be hydrolyzed to AKG. Our recent study has shown that DMAKG significantly promotes osteogenic differentiation and bone regeneration in aged mice. However, its cytotoxicity and rapid hydrolysis limit its application. To address these challenges, we aim to develop a novel system for the sustained delivery of AKG and other drugs directly into cells, investigating its potential for bone regeneration through *in vitro* cell culture and *in vivo* animal studies.

Methods: Specifically, we design and synthesize a novel polyester incorporating an AKG moiety (PAKG) to fabricate microparticles (MPs) with different sizes.

Results: *In vitro* studies demonstrate that PAKG MPs significantly promote osteogenic differentiation indifferent cell types through phagocytosis. Furthermore, we found that size and hydrophilicity of the MPs have a significant impact on their biocompatibility and pro-osteogenic capabilities. Excitingly, these biodegradable PAKG MPs are highly phagocytosable for nonphagocytic cells (e.g., pre-osteoblasts MC3T3-E1 and primary bone marrow MSCs). They significantly promote osteoblastic differentiation and mineralization while common poly (L-lactic acid) and poly (lactic-co-glycolic acid) MPs (PLLA & PLGA) with similar size fail to do so. Notably, even a minimal 1% incorporation of PAKG into PLGA or PLLA MPs enables their efficient phagocytosis. Beyond AKG, these PAKG MPs facilitate intracellular delivery of small compound phenamil, achieving the highest osteogenic differentiation compared to PLGA MPs or soluble format.

Conclusion: While *in vivo* studies are still in process, the novel PAKG-based MPs show great promise to improve osteogenic differentiation and enable efficient intracellular drug delivery through phagocytosis for broad regenerative medicine.

Supported by: NIH R01 DE029159.

Oral Session 7

34. Polydopamine Coating of PLGA for Increased Intracellular Drug Delivery



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Objective: The objectives were to improve the osteoblastic internalization of PLGA microparticle by increasing its surface hydrophilicity and tuning its surface charge using a polydopamine coating, and to evaluate

if the Polydopamine-coated PLGA functioned more efficiently as a drug delivery system.

Methods: All PLGA microparticles were synthesized using a homogenization technique, and some of the synthesized microparticles were bathed in a polydopamine solution for 6 hours. SEM imaging was used to visualize the success of the surface coating. MC3T3-E1 pre-osteoblasts were cultured and split into three different groups: osteoblasts alone, PLGA/osteoblasts, and polydopamine-coated-PLGA/osteoblasts. Light microscopy and confocal imaging was performed on the different groups to show cellular interactions. A pro-osteogenic drug (phenamil) was loaded onto the PLGA microparticle and polydopamine-coated PLGA microparticle. ALP assay was used to measure osteogenic activity of the pre-osteoblasts after seven days of being treated with different microparticles.

Results: The SEM imaging showed surface changes of the

PLGA microparticles after polydopamine coating. The light microscopy and confocal images showed that MC3T3-E1 pre-osteoblasts internalized large amounts of the polydopamine-coated PLGA microparticles but barely internalized any of the uncoated PLGA microparticles. The ALP activity was similar between the Phenamil/PLGA group and the Phenamil/polydopamine-coated-PLGA group. The ALP activity decreased as the concentration of phenamil-polydopamine-PLGA added to the osteoblast cultures increased.

Conclusion: The polydopamine-coated PLGA microparticle had increased cellular interaction and internalization with MC3T3 osteoblasts compared to uncoated PLGA microparticle. However, when the polydopamine-coated PLGA microparticle was loaded with phenamil, its osteogenic activity did not have an apparent increase. Further study needs to be done to determine the potential underlying mechanism.

Supported by: The Department of Oral and Maxillofacial Surgery.

35. Regeneration Potential of Stem Cell Secretome from Different Culture Conditions



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Objective: The objectives were to compare traditional 2D and hanging-drop 3D cultures of hADSC along with comparing two different 3D culture methods: hanging-drop assay vs

38. Porous Chitosan Matrix Decorated 3D-Printed PCL Scaffold for Tissue Engineering



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Objective: 3D printed scaffolds are attractive because of the capability of personalized and accurate control over geometric structure and their scalability. Polycaprolactone (PCL) has been widely used for 3D printed scaffolds by virtue of its excellent biocompatibility in bone regeneration. However, the bioinert nature of PCL and lack of drug loading capacity hinder their application. We recently developed an innovative chitosan-vanillin-bioglass (CVB) 3D porous scaffold with strong osteopromotive and antibacterial abilities as well as high water absorption capacity. We hypothesize that decorating 3D printed PCL scaffolds with porous CVB matrix (CVB+PCL) will take the advantages from both sides.

Methods: CVB+PCL composite scaffolds were prepared by the 3D printing/freeze-drying processes and characterized by SEM and mechanical test. Cells proliferation and osteogenic

differentiation were quantified using MTS assay, ALP activity, and calcium content test, respectively.

Results: Our data showed porous CVB matrix uniformly filled the large pores of 3D printed PCL scaffolds. The “hard” part (PCL) provided good mechanical support, while the “soft” part (CVB) endowed the pro-osteogenic abilities and drug loading capacity. Human gingival fibroblast derived secretome (hGF-CM) was collected and enriched by using its unique ability for fast tissue healing. Our data confirmed that hGF-CM was able to increase the proliferation, migration, angiogenesis, and osteogenic differentiation of stem cells. Furthermore, sustained release of growth factors can be achieved after loading hGF-CM onto CVB scaffolds. The subcutaneous implantation model confirmed that hGF-CM loaded CVB can significantly improve host cell migration to the scaffolds. CVB+PCL together with hGF-CM can synergistically increase stem cells osteogenic differentiation.

Conclusion: Thus, the combination of 3D printing and CVB porous matrix is a promising approach to greatly expand the application of 3D printed scaffolds in tissue engineering and drug delivery.

Supported by: NIH R01 DE029159.

Oral Session 8

39. Patient Assessments of Adjunctive Dissolvable Gels Under Removable Dentures



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Objective: Patients who wear removable dentures have limited options in relieving removable denture-related challenges such as comfort, retention, and xerostomia. Traditional denture adhesives aim to improve comfort and retention but come with the downside of increased hygiene burden due to the difficulty in their subsequent removal. The purpose of this study is to assess the effects of oral gel-based products, MI Paste (GC America) and Biotene Dry Mouth Gel (Haleon), on their off-label uses under removable dentures in improving patient comfort, retention, and xerostomia.

Methods: Patients were screened for adequately fitting removable denture(s), with baseline metrics recorded including use of denture adhesives and xerostomia status. Numerical baseline ratings (1-10) for patient-assessed comfort and retention of their removable denture(s) were recorded. Volumetric syringes of MI Paste and Biotene Dry Mouth Gel were provided for once daily application of 1mL of each product for one week. At the end of each week, telephone interviews were conducted to collect subjective feedback on changes in comfort and retention along with other relevant variables. Improvements in xerostomia and continued utilization of traditional adhesives, if applicable, were recorded. Descriptive statistics were calculated on all metrics.

Results: In the ongoing study, 53 participants have completed both phone call follow-ups (39.6% males, mean age 68.3 years). Of the 37 individuals who reported experiencing xerostomia at baseline, it was observed that MI Paste and Biotene Dry Mouth Gel yielded improvement in 83.8% and 75.7% of cases, respectively. Slight increases (<1 pt) in mean comfort and retention were found with both products. Of the 19 participants who reported use of denture adhesives, 26.3% and 21.1% of them considered MI Paste and Biotene Dry Mouth Gel as an alternative, respectively.

Conclusion: Dissolvable oral gels show promise in offering relief for xerostomia to patients wearing removable dentures. Additional subjective benefits may not be immediately evident.

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

40. Deviation of Fully-Guided Tooth-Supported Surgical Guide: Systematic Review and Meta-Analysis



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Objective: This systematic review and meta-analysis aimed to evaluate the depth distortion and angular deviation of fully-guided tooth-supported surgical guide (FTSG) in partially edentulous arches.

Methods: This study followed the PRISMA guidelines and was registered in the Open Science Framework. The formulated PICO question was “In partially edentulous arches, what are the depth distortion and angular deviation of fully-guided tooth-supported static surgical guides?”. In FTSG, two surgical approaches were compared: open flap and flapless techniques and two digital methods were assessed with fiducial markers or dental surfaces. A qualitative analysis for clinical studies was used to assess the risk of bias. In addition, a meta-analysis was performed to evaluate the angular deviation of freehand and FTSG.

Results: Nine studies reported angular deviations ranging from -0.32° to 4.96°. In terms of surgical approaches, six studies reporting mean angular deviations ranging from 2.03° to 4.23° for the open flap technique. Four studies evaluated flapless, reporting angular deviations ranging from -0.32° to 3.38°. Five studies assessed the freehand surgical approach, reporting angular deviations ranging from 1.40° to 7.36°. The mean depth distortion ranged between 0.19 mm to 2.05 mm for open flap, and between 0.15 mm to 0.45 mm for flapless. In terms of digital methods assessed for surgical guide design with dental surfaces the angular deviations of six studies ranges between 1.40° to 4.96°, and for fiducial markers the angular deviations of two studies ranges between 2.81° to 3.38°. Seven studies were eligible for meta-analysis, heterogeneity was low between studies comparing freehand vs. fully-guided in open flap techniques (I² (95%CI) = 21.3% (0.0% to 67.8%)), favoring the fully-guided approach.

Conclusion: FTSG systems exhibited less angular deviation than freehand and partially-guided surgery techniques. Flapless surgical showed less angular deviation and depth distortion, regardless of the alignment technique (fiducial marker or dental surface).

41. GentleWave Usage Among Practicing Endodontists: A Survey on Treatment Protocols



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Objective: An assortment of endodontic irrigation devices is available to the practicing dentist. Utilization of different technologies might contribute to enhanced disinfection of the root canal system which may improve endodontic treatment outcomes. The GentleWave (GW) is a recently developed multi-sonic irrigation device designed for use after minimal instrumentation. The aim of this study was to investigate the GW treatment protocols of active members of the American Association of Endodontists (AAE).

Methods: A cross-sectional survey involving 37 multiple-choice anonymous questions was constructed and distributed electronically using Qualtrics to active members of the AAE (n=3,610). The survey was available for one month with a reminder email sent two weeks after the initial invitation. Statistical analysis consisted of descriptive and bivariate analyses ($\alpha=0.05$).

Results: A total of 457 participants completed the survey yielding a 12.7% response rate. The analysis included 230

subjects, all with experience using the GW. Nearly 14% of subjects received GW training during their residency program. Among the endodontists currently using the GW (n=177), the majority (52.6%) have utilized it for more than three years, incorporating it into 76% or more of their cases (50.3%). Endodontists using the GW for more than three years were less likely to consider pulpal and periapical diagnosis (24.2% vs 41.5%; p=0.015), tooth type (37.8% vs 53.1%; p=0.045), or complexity of tooth anatomy (52.2% vs 69.1%; p=0.024) when deciding to run the GW. Subjects with experience using the GW system that no longer incorporate it in their practice (n=48) stated cost (72.9%) and time (60.4%) were the most common reasons for discontinuing use.

Conclusion: GW usage patterns are clinician dependent. There is a lack of consensus regarding the optimal method for utilizing the device. Duration of system use significantly impacts the frequency of use.

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

42. Early Stages of a Self-Determination Theory-Based Clinical Trial for ECC-Prevention



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Objective: This research compares the efficacy of autonomy-supportive videotaped oral health messages framed by self-determination theory (SDT) to more traditional neutral messages in the prevention of early childhood caries (ECC). This presentation describes the protocol for a randomized clinical trial and preliminary demographic findings.

Methods: Six-hundred and thirty-four pregnant mothers enrolled in WIC Programs are being recruited and followed until their future children are 36 months old. Pregnant mothers are randomly assigned to either an experimental SDT group where they receive autonomy-supportive messages, or a control group where they receive the same oral health content delivered using a neutral style. All mothers are exposed to a series of three videotaped oral health messages—during pregnancy, and when their future child is 12 and 24 months. Prior to and after watching the videos, participants complete a series of pre- and post-intervention questionnaires. The primary outcome of interest will be children's caries status. Secondary outcomes will be changes in caregiver's behavior towards the child's oral health (e.g., oral hygiene and dietary habits), plaque levels and mutans streptococci counts.

Results: Thus far, 142 subjects completed the initial visit (49% in the experimental group and 51% in the control group). Baseline results showed that participants' mean age was 26.5years; they were most frequently Caucasians (60%), single (55%), employed >32 hours per week (44%), had a high school diploma or GED (66%), and had annual incomes below \$5,000 (23%). Thirteen percent identified themselves as Hispanics. Ninety-six participants completed the 1-month follow-up

Continued ►

questionnaires (54% experimental group), and 74 of them watched the booster video message at the 3-month interval (53% experimental group).

Conclusion: Preliminary results indicate recruitment from a sample of WIC-enrolled low-income pregnant mothers with good minority representation. If the trial is successful, SDT could be applied in high-risk populations to alleviate the ECC-burden.

Supported by: NIDCR UH3 DE029443.

43. Risk of ER Visits After Dental Treatments in Older Adults



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Objective: With the rising prevalence of dental care needs in the elderly population, there is a growing concern about the risks associated with providing dental care to

older adults who often suffer from significant comorbidities or frailty. Invasive dental treatments (IDTs), characterized by aggressive and complex procedures, may induce stress and anxiety, potentially exacerbating existing comorbidities or leading to complications requiring emergency room (ER) visits.

Our study investigates the association between IDTs and the development of complications necessitating ER visits within 7 days post-treatment in older adults.

Methods: This retrospective cohort study included 2,567 adult patients aged over 65, drawn from UI EPIC and Axiom databases. Eligible participants were patients of COD and UIHC who underwent dental extractions, as indicated by CDT codes. We defined our outcome as whether patients required an ER visit within 7 days of treatment. Statistical analyses included the Wilcoxon rank-sum test for continuous variables and the chi-square test for categorical variables. A multivariate logistic regression analysis adjusted for age, sex, race, insurance status, and comorbidities was conducted.

Results: Our multiple logistic regression model, including age, sex, race, insurance status, cardiovascular diseases, pulmonary diseases, and diabetes, identified significant associations with ER visits post-IDT. Notably, age ($p=0.003$), sex ($p<0.001$), insurance status ($p=0.023$), cardiovascular diseases ($p<0.001$), and diabetes ($p=0.028$) were significant predictors. The receiver operating characteristic curve for this model showed an area under the curve (AUC) of 0.701 (95% CI: 0.657-0.744, $p<0.001$).

Conclusion: Our study highlights key factors contributing to ER visits following IDT in older adults. These findings can aid dentists in clinical decision-making, fostering safer and more effective treatment planning for this vulnerable population.

hypochlorite solution (NaOCl), PA gel, SDF 5) SDF, potassium iodide (SSKI) 6) PA gel, SDF, SSKI.

X-ray diffraction (XRD) was completed at baseline, immediately after application protocols, and following an aging protocol through storage in artificial saliva for 1 week and wear testing with a toothbrush simulator. Scanning electron microscopy was completed at baseline and at the conclusion of testing. The color appearance of each sample's surface was recorded at each stage.

Results: Chemistry and color of precipitates varied across groups immediately after protocol applications, although all groups resulted in dark colored precipitates after aging. Chlorargyrite (AgCl) was identified in all aged groups, although silver iodide (AgI) precipitates in Groups 5 and 6 remained stable following simulated aging.

Conclusion: The results suggest ongoing research on the methodology of SDF application protocols to strengthen caries management outcomes. Refining protocols to ensure the highest concentration and deepest penetrating silver and fluoride precipitates should continue to be sought regarding SDF's application methodology, although preventing the darkening of carious tooth structure appears evasive.

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

45. Trainees Experience With a Poverty Simulation



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Objective: Health disparities disproportionately affect vulnerable populations, particularly the poor and elderly.

The purpose of this longitudinal study is to assess shifts in the attitudes of University of Iowa Leadership Education in Neurodevelopmental Disabilities (LEND) students, nursing students, and faculty/ staff regarding treating underserved populations based on an educational model that incorporates a poverty simulation as component of their education.

Methods: A total of 410 LEND and nursing students were invited to participate in a poverty simulation between the years of 2019-2023. A 20-question survey measuring participants' beliefs regarding poverty on a 5-point Likert scale was administered immediately before and after the simulation. The responses from both surveys were paired to evaluate changes in beliefs. One point was assigned for each empathetic response (maximum positive score=20), while negative belief responses were scored zero. Statistical analysis included Bowker's symmetry test and weighted Kappa statistic ($\alpha=0.05$).

Results: The study comprised 385 participants who completed both pre- and post-surveys. The average age of participants was 24.0 ± 7.2 years, 34.7% LEND students, 11.2% as males, 80.7% Caucasian, and 88.4% expressing moderate-liberal political beliefs. Shifts in responses between pre- and post-surveys were observed for sixteen questions ($p<0.05$ in each instance). Notably, 201 subjects (52.2%) responded empathetically to 16 or more of the 20 questions prior to the simulation while 288 (74.8%) provided empathetic responses to 16 or more questions after the simulation. Levels of agreement ranged from kappa=0.62 to kappa=0.16 indicating that changes of opinions about poverty occurred after participating in the poverty simulation.

Conclusion: Engaging in a poverty simulation can educate future healthcare professionals about the challenges faced by patients living in poverty but also foster the development of empathy towards these individuals.

Supported by: University of Iowa College of Dentistry Department of Pediatric Dentistry.

46. Oral Microbiota Dynamics and Metabolomic Shifts in Fixed Orthodontic Therapy



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Objective: With orthodontics gaining popularity for enhancing esthetics and function, approximately 4.5 million Americans, predominantly aged 6 to 18, wear braces. Fixed appliances impair efficient oral hygiene and heighten the risk of caries and gingival disease. Our objective is to employ a comprehensive, integrated -omics approach to assess changes in the microbial environment, host inflammatory interactions, and end-stage molecules (metabolites) within the subgingival environment of patients undergoing fixed orthodontic therapy (FOT).

Methods: This cross-sectional analysis utilized data from an ongoing longitudinal study of 69 periodontally healthy, non-smoking individuals (ages 11-24), with subjects matched for age, gender, and dentition across four groups: Control (no orthodontics), and those undergoing 6-, 12-, and 24-month orthodontic therapy. Subgingival plaques were collected, bacterial DNA isolated, V3-V4 regions amplified, and 16S sequencing performed on the Illumina Miseq 2x300bpPE platform. Sequences were annotated against the SILVA database. Multiplex assays quantified adipokines and cytokines; significance was determined by Tukey HSD. Metabolite peaks from Gas Chromatography/Mass Spectrometry were annotated against the Small Molecule Pathway Database, with significance assessed through enrichment analysis.

Results: FOT was associated with a significant reduction in bacterial diversity compared to controls; however, the diversity was similar among different time points. *S. mutans* was significantly increased in all FOT groups when compared to controls. In addition, FOT exhibited an increase in several caries and inflammation-associated species belonging to Rothia, Prevotella, Saccharibacteria, and Selenomonas compared to controls. Interestingly, metabolites that have been previously demonstrated to play a role in bone homeostasis and collagen maturation, such as cysteine, homoserine, and beta-alanine, are increased in the later FOT time points, while metabolites associated with inflammatory regulation, such as hypotaurine and phenylalanine, are increased at 6-months.

Conclusion: Our integrated -omics approach is the first-step towards understanding the comprehensive interdependent roles of oral microbiota and host signals of bone homeostasis and inflammatory regulation during FOT..

Supported by: University of Iowa College of Department of Periodontics.

Posters

44. Silver Diamine Fluoride Preliminary *In Vitro* Investigation of Application Protocols



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Objective: Silver diamine fluoride (SDF) has garnered significant interest relating to its use in caries management. Further investigation is indicated regarding the type, concentration,

and stability of SDF precipitates formed after its application clinically. An *in vitro* study conducted aimed to evaluate the precipitates formed following SDF's application under various protocols.

Methods: Eighteen root dentin blocks (4x4mm) were obtained from the cervical third of human premolars and embedded in epoxy resin and polished. Artificial carious lesions were created using a pH-cycling protocol (14 days). Six experimental SDF (38%, Advantage Arrest®) application protocols were designed investigating supplemental application of other currently available solutions (20 second application and rinse) with three samples assigned to each group. Group assignments included: 1) SDF 2) SDF, fluoride varnish (5% NaF) 3) 35% phosphoric acid (PA) gel, SDF 4) 3% sodium

47. Intention of Community Health Center Dentists to Remain at CHC



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Objective: Access to dental care is not uniform throughout the United States; these disparities can be seen through various demographics.

With fewer dentists pursuing careers post-graduation in public health sectors, as well as reports of increasing burnout, it is imperative to understand how to recruit and retain dentists to high needs areas to address access to care challenges. There is limited research which examines characteristics of dentists associated with long-term practice in underserved communities. The purpose of this study was to identify factors related to dentists’ intent to continue to practice in a community health center (CHC) long-term.

Methods: Data were from a 2021 workforce survey conducted by the National Network for Oral Health Access (NNOHA), a national organization for U.S. safety-net oral health practitioners. The dependent variable was dentists’ intention to remain in practice at their current CHC for more than 7 years. The 42 independent variables were categorized into one of the following domains: organizational, individual perceptions, individual motivators, and individual characteristics. Descriptive analysis and chi-square tests were completed.

Results: The final sample included 248 dentists. Respondents with less than 10 years of practice experience were significantly associated with an intention to leave in less than 7 years (p=0.005), as were dentists in leadership positions (p<0.001), and low to moderate perceived professional autonomy (p=0.002). Altruistic motivation to practice in a CHC was not significantly associated with intention to remain long-term (p=0.995) nor was a desire to practice in a community-based setting (p=0.909).

Conclusion: Factors significantly related to intention to remain at their current CHC long-term included individual perceptions and individual characteristics, while organizational characteristics and individual motivation were not significantly associated with intention to remain. Further research is needed, including prospective cohort studies, to provide more comprehensive insight into factors related to retention of dentists at CHCs.

48. Reasons for Failed General Anesthesia Pediatric Dental Procedures



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Objective: Failure to attend a general anesthesia (GA) appointment can be costly, inconvenient, and hazardous to the health of the patient. The purpose of this study is to evaluate reasons pediatric dental patients

failed GA appointments. This was a follow-up study to a similar study completed in 2021, aiming to build upon and further investigate the insights obtained from the earlier research.

Methods: Using methods from the previous study, data were collected using a retrospective review of 276 charts of patients with GA appointments at a university pediatric dental clinic between September 26th, 2022, and May 31st, 2023. Information obtained included age, sex, distance between residence and the university, insurance type, referral status, affiliation of primary care provider, special health care needs (SHCN) status, completion of intermediate dental treatment, time between consultation and GA appointment, and reasons for failed appointments. Descriptive and bivariate analyses (alpha = 0.05) were conducted.

Results: 276 patients were included in the study. There were 45 failed and 231 kept appointments. Subjects who received emergency dental care between consultation and the GA appointment were more likely to fail than those who did not (26.5% vs. 14.1%; p=0.033). Subjects with SHCN were less likely to fail than those without (11.8% vs. 20.7%; p=0.044). The mean/ median days between consultation and the GA appointment for subjects who failed were significantly higher than for those who kept their appointment (219.5/215.5 vs. 166.8/181; p=0.002). Factors of age, sex, distance between residence and the university, insurance type and clinical variables showed no significant associations with failed appointments.

Conclusion: Significant differences were found between patients who failed their appointments compared with patients who kept their appointments if they received emergency dental care and were a patient with SHCN.

Supported by: University of Iowa College of Dentistry Department of Pediatric Dentistry.

49. Mouthguard Use by School Aged Children: A Survey



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Objective: Sports injuries account for up to 40 percent of all dental injuries in children. The American Academy of Pediatric Dentistry encourages dentists to play an active role

in educating the public on the use of mouthguards for prevention of dental injuries. Although several studies have emphasized the benefits of mouthguards for the prevention of dental injuries, many young athletes still choose not to use mouthguards.

The purpose of this study is to investigate if athletes wear mouthguards, identify the potential barriers, and educate parents on the importance of mouthguards in preventing dental sports injuries.

Methods: A 15-question survey was administered to the parents/guardians of school-aged children who reported participating in sports. The study population consisted of 93 parents/children, 60 males (66%), 31 females (34%). Survey questions included the sport(s) the child participated in, if a mouthguard was worn, and specific reasons that impacted if a mouthguard was utilized or not. Specific factors impacting mouthguard use included cost, social pressure, comfort,

performance, coach requirement, and dentist recommended, as well as the level of importance of each.

Results: The sport with the highest reported use of mouthguards was football (81%), followed by wrestling (41%), basketball (16%), and soccer (11%). Parents/guardians ranked “required by coach” was the strongest factor in determining mouthguard use (73%). The second strongest factor was “recommended by dentist” at 70%. Eighty-eight percent strongly agreed/agreed “mouthguards play an important role in protecting against dental trauma injuries”. Eighty percent reported they are more likely to have their child wear a mouthguard after completing this survey.

Conclusion: Children are more likely to wear mouthguards in sports when it is required by the coach and/or recommended by a dentist. It is imperative to educate coaches and dentists on the use of mouthguards in school-aged children for the prevention of dental injuries.

50. Post Surgical Recovery in Dental Patients With and Without Frailty



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Objective: Frailty is a common geriatric syndrome characterized by age-associated declines in physiologic reserve and function across multiorgan systems, leading to

increased vulnerability for adverse health outcomes after extensive surgery. However, how frailty affects post-surgical recovery in geriatric dental patients remains unclear. This study was to examine the feasibility of a study protocol that aims to investigate whether dental surgery may increase the risk of developing geriatric syndromes and other complications within 1-7 days after invasive dental treatment in older adults aged ≥ 65 years old with frailty.

Methods: A convenient sample of 6 older adults aged ≥ 65 years old undergoing tooth extraction(s) were recruited from the University of Iowa College of Dentistry and Dental Clinics. After informed consent, eligible participants completed an assessment of frailty and proceeded to the proposed extraction(s) with their dentists or dental students. Besides pain, bleeding, and infection at the surgical site, other outcome measures (e.g., disorientation, dehydration, etc.) were to be assessed on the surgical day and 1 day, 3 days, and 10 days after dental surgery. Participants were interviewed at the end of the study to assess the feasibility of the study protocol.

Results: The feasibility interviews showed high satisfaction with the study and there were few recommendations for improvement. Participants reported that they thought the questions were easy to understand, and that phone calls were a convenient way to participate in the study. Suggestions included having more coordination with the clinic as multiple participants would ask questions related to their dental care. Participants also conveyed that the primary barrier they experienced for participation in the study was coordination of travel with a caregiver.

Conclusion: After considering the feedback from pilot participants, we have determined that this study protocol is feasible to implement in the University of Iowa College of Dentistry and Dental Clinics.

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

51. Urgent Care Teaching at University of Iowa College of Dentistry



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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 survey questionnaire was distributed via email through REDCap to current faculty, adjunct faculty, and residents at the University of Iowa College of Dentistry (n=401). The participants were asked to indicate if topics of a typical dental urgent care appointment should be included in the dental 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 63 (15.7%). Twenty topics were surveyed and 33% (n=21) of respondents were General Dentists. The other 67% indicated that 11% (n=7) had completed an Advanced Education in General Dentistry, 11% (n=7) in Dental Public Health, 4.8% (n=3) in Endodontics, 6.3% (n=4) in Operative Dentistry, and 9.5% (n=6) in Pediatric Dentistry. More than 90% of participants spent less than 50% of their clinic time in dental urgent care. Among the possible topic selection, there was a relative uniformity on 8 topics that all respondents believed should be included in the urgent care curriculum. These 8 topics each had a median rank of importance between 8 and 10. The other 11 topics ranged from 64-97% of the respondents believing they should be included in the curriculum, with ranked importance ranging from 6 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.

52. Fad Diets Can Increase Risk of Oral Disease



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Objective: Fad or unconventional diets are popular and heavily promoted on social media. However, it is unclear how fad diets might impact oral health.

Our objective was to identify the fermentable carbohydrate exposures, nutrient adequacy, and acid exposures associated with fad diets to determine their potential caries, periodontal disease, oral cancer, and erosion risk.

Methods: Ten popular diets promoted on social media were selected. Information regarding the food profile (i.e., foods allowed or disallowed) and the meal pattern of each diet were obtained from internet sites. The food profiles and meal patterns were evaluated to identify probable fermentable carbohydrate exposures, nutrient adequacy, and acid exposures and how these might contribute to caries, periodontal disease, oral cancer, and/or erosion risk.

Results: Caries risk is likely increased with the Master Cleanse diet, which recommends a sugared lemonade beverage 6-12 times/day. Periodontal disease risk is likely increased with the Ketogenic, Master Cleanse, Grapefruit, Cabbage Soup, and Raw Food diets, all of which are nutritionally inadequate, and slightly increased with the SiOBeauty, Paleo, and South Beach diets, which are marginally adequate. Oral cancer risk is likely increased with the Ketogenic, Master Cleanse, and Grapefruit diets, which are limited in fruits and vegetables, and slightly increased with Paleo diet, which is limited in some fruits and vegetables. Lastly, erosion risk is likely increased with the Master Cleanse and Grapefruit diets, which both include significant citrus exposures. The Mediterranean and Gluten Free diets are not associated with increased risk of oral disease.

Conclusion: Oral health care professionals should be cognizant of popular dietary trends as a patient's diet can impact their oral disease risk. Potential risks of caries, periodontal disease, oral cancer, and erosion can be identified from a diet's probable fermentable carbohydrate exposures, nutrient adequacy, and acid exposures.

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

53. Optimizing mRNA Preservation In Laser Microdissection-Coupled RNA-Seq



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Objective: In a developing tooth, signaling between Hertwig's Epithelial Root Sheath (HERS) and adjacent mesenchyme regulates root development. HERS develops at around P7 in mice. Laser microdissection (LMD) and SmartSeq2 have been used to generate transcriptome profiles of cells from embryonic tooth sections. However, the standard tissue processing protocol for adult teeth, which includes a

decalcification step, severely damages RNA integrity. This study aimed to optimize the tissue processing protocol of P7 and P10 mandibles to preserve RNA, ultimately enabling generation of RNA-seq libraries.

Methods: 5 P7 and 7 P10 wild-type mouse mandibles were fixed in methacarn for 6 hours and decalcified in various solutions for various duration. Mandibles were embedded in paraffin and sectioned at 7 microns. First molar HERS and mesenchymal cells were collected via LMD. SmartSeq2 protocol produced RNA-seq libraries; adjustments to SmartSeq enzyme volumes were also tested. qPCR, Qubit, and bioanalyzer assay were used to analyze concentration and integrity of libraries.

Results: We found that the optimized decalcification protocol for both P7 and P10 was 20 hours decalcification in RNA Later with 15% EDTA. RNA Later helped preserve RNA. Lower EDTA concentrations resulted in greater section breakage and poor RNA integrity; 15% was the highest attainable concentration in RNA Later. Though shorter durations yielded viable sections for P7, it did not improve RNA integrity, and sections were more prone to breakage. A longer decalcification time would be preferred for easier sectioning of P10, but RNA integrity was compromised past 20 hours decalcification. Doubling all SmartSeq2 reaction volumes recovered a greater amount of RNA; increasing RNase inhibitor alone did not preserve greater RNA integrity.

Conclusion: We found an optimized tissue processing protocol that enabled us to generate RNA-seq libraries for adult teeth. This protocol guided further experiments into the mechanisms of HERS and root development.

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

54. Identifying Strategies to Reduce Food Insecurity Among Dental Students



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Objective: Food insecurity (FI) among post-secondary students has received little attention despite its high prevalence. FI is a barrier to student success and increases the risk of

health-related issues, yet university/community resources to address FI are underutilized. Our objective was to identify strategies to address FI at Iowa's College of Dentistry.

Methods: Dental students (n=328) were recruited via email to complete an online survey querying demographics, food security (FS) status, perspectives of available food resources, and strategies to reduce FI. The survey was open for three weeks, and participants were compensated with a \$15 gift card.

Results: Participants (n=174) were 66.1% female, and 32.2%, 29.9%, 24.7%, and 13.2% were first, second, third, and fourth-year students, respectively. Nearly a quarter (23.5%) reported FI. While 41.1% of all students reported insufficient time to purchase food as their greatest barrier, 78.3% of FI students reported insufficient money. FI students reported

less community access to preferred foods than FS students (p<0.001), while nearly a fifth (19.5%) of all students reported food restrictions prevented eating at collegiate events. Student suggestions to address this barrier included increasing lactose/dairy-free, meat-free, gluten-free, and carb-free options, and labeling allergens. Over half (55%) of students were unaware of food assistance programs with 42.0% unaware of university/community food pantries. Among 94.8% not using food pantries, 12.8% did not know their location and 30.5% reported "others needed the resources more." FI students were less aware of resources than FS students (p=0.01). Students supported collegiate grab-and-go breakfasts/lunches and grocery gift cards from survey strategies to address FI. Student-suggested strategies included cheaper and healthier cafeteria items, mandatory lunch breaks between clinics, and additional lunch-and-learns.

Conclusion: Lowering financial barriers to healthier and more diverse food options may be the most effective to address FI in dental students. Increasing awareness and addressing stigma towards existing resources is also necessary.

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

55. Associations Between Water Insecurity and Beverage Intakes in Pediatric Patients



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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 demographics, perception of water security, and beverage intakes (Child's beverage Intake Questionnaire).

Results: Children (n=44) were 5.6±1.3 years old; 56.8% White, 20.5% Black/African American, and 18.2% Multiracial/Other; and 23.3% Hispanic or Latinx. The majority of respondents reported their highest education level was 8th grade or less (30.23%); the next highest level was a college graduate (27.9%). Regarding tap water, 70.4% of respondents either strongly or somewhat agreed that their local tap water was safe to drink. For bottled water, 54.5% of respondents agreed that bottled water is safer than tap water, with 34.1% neither agreeing nor disagreeing with that statement. The association between children's tap water intake and intake of SSBs was not statistically significant (p = 1.0).

Conclusion: The majority of respondents consider bottled water safer than tap water. Preliminary results do not support

an association between tap water and SSB intakes; continued investigation is necessary.

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

56. Factors for Online Dental Portal Use



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Objective: To investigate the characteristics of patients enrolling in the online dental patient portal (PP) at the University of Iowa College of Dentistry (UICOD) and compare to patients who

do not use the PP.

Methods: A retrospective chart review of health histories and consents was conducted for new patients at the UICOD January, 1 2022 through May 31, 2023 seen in the Admissions, Pediatrics, and Family Dentistry clinics. PP (n=434) users were matched based on age, size of community, and clinic assignment to a non-user patient. Gender, distance to the UICOD, insurance, completeness of online forms, and clinic for next visit were evaluated. Conditional logistic regression was used to examine potential associations between portal use and the covariates gender, insurance status, referral status, distance to the clinic, and clinic for treatment during the next visit. Any evidence of such associations would indicate factors that influence patients' completion of their consents/histories through the PP.

Results: A total of 897 charts were reviewed with a median age of 24 and distance of 56 miles. Sixty-eight percent of patients were from populations of greater than 50,000 people and 99 percent of patients indicated English as their preferred language. Among PP users who completed a consent form on/ before their appointment, all consents and health histories had completion rates higher than 89 percent. Preliminary results showed that greater distances traveled and no referral were associated with lower odds of using the PP.

Conclusion: The data suggests that traveling from further distances was associated with decreased odds of completing forms on the PP before their first visit while a referral is associated with increased odds of completing forms. All other variables investigated were not significant for PP use.

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

57. Self-Report of Difficult to Anesthetize—
Patient and Provider Review



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Objective: The frequency of local anesthesia inadequacies in dentistry is poorly defined, with a potential separation of observed outcomes between a patient and their provider.

The contributing role of a provider or the impacts of the type of care being delivered on tough to numb (TTN) reports are indicated for further investigation. The study's objective was to enhance understanding of potential associated factors in patients self-reporting to be TTN through an email survey and a retrospective review of patient electronic health records (EHR).

Methods: A 22-item survey invitation was emailed to 508 patients at the University of Iowa College of Dentistry (UICOD) who indicated being TTN in their dental history forms. Additionally, a retrospective EHR review of TTN patient procedure notes entered by providers was completed to assess reported treatment tolerance and local anesthesia practices utilized. Descriptive and bivariate analyses were conducted (alpha=0.05).

Results: Fifty-three subjects (73% females, 13.2% natural red hair) completed the survey with a response rate of 10.4%. Sixty-eight percent reported having difficulty becoming fully numb for dental procedures either always or most of the time, with 34% reporting only in certain anatomical areas. Six-hundred and sixteen EHR procedure notes of 195 patients at UICOD self-reporting being TTN (64% females, mean age of 41.6±14.9 years) were analyzed. About seven percent of procedural notes had recorded patients being TTN, while 6.5% of notes had recorded anxious patient behavior. The local anesthetic articaine was used in 67.2% of procedures, highest in endodontic procedures (83%), and only 21% of procedures involving mandibular teeth.

Conclusion: The results reveal several complexities relating to TTN reports. Improving demographic identification and procedural documentation along with their respective outcomes will better define evidence-based practices in managing and preventing negative dental experiences in TTN patients. Further statistical analysis is ongoing.

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

58. Perceived Social Status and Mental
Well-Being Among Pre-Doctoral Dental
Students



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Objective: Perceived Social Status (PSS), a subjective measure of one's social position, has been associated with multiple health outcomes. Previous research demonstrates that elevated levels of stress, depression, and anxiety are all prevalent among pre-doctoral dental students, but the role of PSS among this population remains unknown. This study investigates the relationship between PSS and mental health among pre-doctoral dental students.

Methods: In Fall 2023, an online survey was distributed to all currently enrolled pre-doctoral dental students at the University of Iowa College of Dentistry & Dental Clinics (UICOD) (N=326). Questions included demographic characteristics, perceived stress, perceived anxiety, and PSS relative to peer community (UICOD) and society. Univariate and bivariate analyses were completed.

Results: The adjusted response rate was 25% (n=83). A majority were female (75.3%) and in their second (24%) and third year (38%) at UICOD. The mean societal PSS (range 1-10) was 6.4 (SD 1.3) and the mean UICOD PSS (range 1-10) was 6.0 (SD 1.7). Comparing societal PSS and UICOD PSS, 45% decreased from societal to UICOD, 33% reported the same PSS, and 23% increased from societal to UICOD PSS. 70% of respondents reported moderate stress (n=58) and 8% (n=7) reported high stress. Sixty-five percent reported minimal-mild anxiety; the remaining 35% reported moderate-severe anxiety. Perceived stress and UICOD PSS have a statistically significant linear relationship (r=-0.320, p =0.003).

Conclusion: Pre-doctoral dental students perceive their social status as lower relative to peers than society (0.36 points lower on average). The fast-paced, highly competitive educational cultures may contribute to mental well-being, but more research is required to better understand these results.

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

59. Salivary Metabolome of Patients Undergoing
Orthodontic Therapy



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Objective: Over half of the 13 and 14 y/o adolescents in the US undergo fixed orthodontic therapy (FOT). Despite acknowledged benefits, concerns persist about the susceptibility of FOT patients to adverse oral outcomes, including compromised oral hygiene, heightened caries risk, gingival recession, and potential periodontal attachment loss.

Emerging evidence indicates disruptions in the oral microbial environment, particularly in the initial weeks of orthodontic treatment.

Methods: Seventy-five participants aged 12–18 undergoing FOT were categorized into baseline (no appliances), 6-month, 12-month, and 24-month groups. Unstimulated saliva samples were collected and stored at -80°C. Gas Chromatography/Mass spectrometry facilitated metabolite analysis, with peaks annotated against the Small Molecule Pathway Database and significance determined using enrichment analysis.

Results: Principal component analysis revealed distinct metabolite clustering based on orthodontic braces' presence and treatment timeline. Metabolites associated with bone remodeling (proline, tyrosine, and citrulline) increased at 6 and 12 months, while those linked to osteoclast functions (lauric acid) surged at 24 months. Several metabolites crucial for mucosal-microbial homeostasis (Indoleacetic acid, taurine) exhibited significant differences between groups, particularly enriched in FOT cohorts. Interestingly, all of these changes were evident even in the absence of any clinical disease, demonstrating the specific metabolomic profiles associated with specific time points of orthodontic therapy.

Conclusion: Our study unveils intricate metabolomic changes induced by FOT, and these findings serve as a foundation for understanding the underlying molecular markers of bone homeostasis in adolescents.

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

60. Silane and Surface Treatment Effects on
Polymer Bond Strength



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Objective: The objective of this in-vitro study was to evaluate the influence of silane-coupling agents on a polymer-based CAD/CAM material containing 80% of nano-ceramic components (zirconia and silica particles).

Methods: A polymer-based CAD/CAM block was halved using a cutting device and then surface-smoothed with a sequence of SiC papers. Various treatment protocols were applied to the surfaces; including airborne-particle abrasion with Al2O3 at 2 bar pressure and 50um particles (APA); 9% hydrofluoric acid for 60 seconds (HFA); a combination of APA and HFA (APHFA); and APA combined with Monobond Etch and Prime following the manufacturer's instructions (APMEP). Silane was not applied to the APMEP group, and for the silane groups, APMEP was omitted. A one-step adhesive was applied to the treated surfaces, followed by the layering of 5mm composite resin in 1mm increments, each cured for 20 seconds. Specimens were cut into 2mm beams and adjusted to 0.8mm round barrel shape using CNC machine. Micro-tensile bond strength (uTBS) testing was conducted at 0.5mm/min on Universal Testing Machine, and results were recorded in MPa. Statistical analysis was performed to assess data distribution and group comparisons. Additional specimens were fabricated for interface evaluation using Scanning Electron Microscopy (SEM) and Confocal Microscopy.

Results: TBS results ranged from 44.47 (±26.12) MPa for APHFA with silane application to 63.72 (±17.14) MPa for HFA also with silane application. On SEM photomicrographs more porosity was present on the interface when a combination of surface treatment (APHFA) was performed. Confocal microscopy showed deeper penetration of adhesive/silane on the same conditions seen on SEM.

Conclusion: A more porous surface is present when combined surface treatments are employed. The adhesive strategy employed on the surface is dependent to the surface treatment. Silane application may not be required when the surface is subjected to airborne-particle abrasion.

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

61. Atomic Scale Analysis of Bone Sialoprotein-
Collagen Interactions



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Objective: Bone sialoprotein (BSP) is a multifunctional extracellular matrix (ECM)

protein within bone, dentin, and cementum. BSP ablation results in bone mineralization deficiencies, reduced acellular cementum, and periodontal degradation in mice. BSP contains three key functional domains: collagen-binding, hydroxyapatite-nucleating, and integrin-binding (RGD) domains. While the N-terminal location and hydrophobic nature of the collagen-binding domain have been elucidated, the precise binding location of BSP on collagen remains elusive. This study aims to explore the feasibility of mapping BSP's binding locations on collagen using atomic force microscopy (AFM) and other methodologies.

Methods: AFM was applied in a stepwise approach to optimize collagen and BSP visualization using three sample groups: collagen, BSP, and BSP bound to collagen. Samples were prepared at 4°C to prevent collagen fibrilization. Samples were imaged using a multimode AFM system with JV scanner and Nanoscope IIIa controller capable of 1 nm resolution, able to measure binding down to ~4 amino acids (aa) within the ~1,400 aa length of collagen. Comparative analysis was conducted between these results and previous atomic visualization techniques (e.g., TEM rotary shadowing) performed in a collaborating lab.

Results: Initial findings indicate promising advances in visualizing collagen-BSP atomic-scale interactions. Early investigations suggest the presence of multiple BSP binding sites on collagen, a previously unreported discovery. Imaging also suggests that BSP binding may alter the structure of collagen fibrils. These results, although promising, require additional replication and optimization for definitive conclusions.

Conclusion: Early stage imaging of BSP-collagen binding interactions reveals promising novel insights. The identification

Continued ►

of multiple binding sites would represent a novel discovery that could enhance our comprehension of hydroxyapatite nucleation on collagen during bone and tooth formation. Further investigations employing techniques such as Toolkit peptides and using mouse and cell models with disrupted BSP-collagen binding will deepen our understanding and insights derived from this research.

Supported by: NIDCR R01 DE027639, T32 DE014320, F30 DE030358; University of Iowa College of Dentistry Student Research Program.

62. Exploring TFAP2-Mediated Indirect Regulation of Odontogenesis via Skin Epithelial Pathways



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Objective: The gain, loss, or misplacement of teeth, whether in syndromic or non-syndromic congenital anomalies, can significantly affect individuals. Understanding early tooth initiation offers pathways for predicting and improving these issues. Tooth initiation is characterized by the formation of a thickened strip in the oral epithelium, referred to as the dental lamina (DL). Despite numerous mouse models highlighting genes in tooth development, most do not impact the initial DL formation. We observed that the early loss of two TFAP2 paralogs, TFAP2A and TFAP2B, before DL formation, is associated with duplicated incisors, although the underlying mechanism remains unknown.

Methods: Here, we test the hypothesis that TFAP2 restricts the expansion of an oral epithelial program by promoting a surface ectoderm program. Thus, in TFAP2^{ECT} mutants, we predict an expansion of the oral epithelial program, concomitant with an expanded DL, leads to ectopic incisors. To test this hypothesis, we use conditional mouse genetics to delete both *Tfap2a* and *Tfap2b* in the ectoderm and collect embryos at key embryonic stages. Embryos are examined using histology, anti-sense RNA in situ hybridization, and single cell RNA-sequencing to investigate changes in the expression of key oral/DL and surface ectoderm defining genes. To further test this hypothesis, we are currently manipulating cell signaling pathways with exogenous ligands to determine their impact on both epithelial and mesenchymal programs during odontogenesis.

Results: In TFAP2^{ECT} mutants, compared to controls, findings from in situ hybridization and single-cell RNA sequencing indicate an enlargement of the oral/DL epithelium at the surface ectoderm's cost. These epithelial alterations in gene expression were associated with parallel changes in the underlying mesenchyme, leading to an increased presence of the odontogenic-related genes.

Conclusion: Collectively, these studies will advance our understanding of how distinct epitheliums in the orofacial region initiate or repress dental identity and the role of TFAP2 in this process.

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

63. Associations Between Food Security Status and Planned Restorations



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Objective: People with low socioeconomic status (SES) are at increased risk for dental caries, and low SES individuals are often food insecure. The purpose of this study was to assess the relationship between food insecurity and caries risk.

Methods: University of Iowa electronic dental records (Axium) were used and data were obtained on patients who were 18+ years who completed their initial CRA and answered food security screener during June 1, 2021-June 1, 2023. Specific planned restorative procedure codes from approximately the same time ($\pm$ 6 months) as the CRA were collected as a proxy measure of caries. Patients were grouped as either "food secure" or "food insecure" based on their responses to the food security screener. Bivariate Chi-Square tests were used to compare planned treatments between those with and without food security and to compare CRA-reported dietary habits based on food security status.

Results: The sample included 5584 participants who were primarily females (58%), and on public insurance (50%), with median age of 41 years. Results were that those with food insecurity were significantly ($p < 0.01$) more likely to have planned restorative treatment than those with food security (74% vs. 61%). Food insecure individuals were significantly more likely to have unstructured meals and consume sugar beverages.

Conclusion: Our findings suggest that individuals with food insecurity are at higher risk of caries than food secure individuals, which may be due to poor dietary habits.

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

64. Factors Associated With Incomplete Primary Endodontic Treatments: A Retrospective Study



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Objective: The reasons for and outcomes of teeth with incomplete primary orthograde root canal treatment (RCT) have not been extensively studied. Phase one of this study aimed to investigate tooth and patient characteristics linked to RCT initiation without subsequent completion in an academic setting.

Methods: Data were collected through electronic dental records at the University of Iowa College of Dentistry (UICoD) from July 2009 to July 2022. Variables of interest included patient demographics and pertinent clinical characteristics.

Results: A total of 291 subjects (one tooth per subject) met the inclusion and exclusion criteria. The average subject age was 39.7 ± 18.1 years (94.5% aged 18 or older). About 69% of subjects were female, 71.5% were white, and 69.4% lived within 60 miles of UICoD. Roughly half of the subjects were on Medicaid insurance. Final clinical designations included incomplete RCT (59.1%), non-restorable designation from treating clinician (22.3%), extraction for unknown reasons (6.9%), and extraction due to patient preference (5.2%). At the last follow-up (868.2 $\pm$ 1252.3 days), nearly 43% of teeth survived while others were extracted (41.8%) or planned for extraction (15.3%). Pulp necrosis (43.5%) and symptomatic apical periodontitis (50.9%) were the most common pulpal and periapical diagnoses, respectively. Nearly 60% of teeth with incomplete RCT were molars and approximately 60% of cases were treated by pre-doctoral students.

Conclusion: Teeth with pain at diagnosis, molar teeth, and treatment performed by novice clinicians may increase the risk of incomplete RCT. Upon completion of final data collection, further statistical analyses will proceed.

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

65. Perceptions on Fluoride and Tap Water Intake: Adults in Alabama



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People's knowledge, attitudes, and behaviors, including their perceptions about fluoride and tap water intake, are important determinants of oral health, but relatively little is known about them.

Objective: To investigate knowledge, attitudes, and behaviors concerning fluoride and tap water intake of adults living in Green-Hill, Alabama.

Methods: A convenience sample of 147 adults aged 18+ with homes on Green-Hill public water completed online questionnaires through Qualtrics. Questionnaires assessed knowledge, attitudes, and behaviors concerning fluoride, as well as tap water intake and demographics. Subjects were divided into regions based on the level of fluoride in the water at their residence, either 0.7mg/L, 0.35mg/L, or 0.1mg/L. Descriptive and bivariate analyses were conducted (chi-square, Kendall's tau) to assess associations between age, gender, and level of education and knowledge, attitudes, behaviors, and tap water intake.

Results: Subjects' mean age was 44.2 ± 15.6 years and 88% were female. Ninety-two percent agreed that fluoride prevents cavities, 84% considered fluoride presence when selecting toothpaste, and 67% drank mostly/all bottled water while at home. Type of water drank at home was not significantly associated with education level ($p = 0.061$). Age was associated with knowledge of the benefits of fluoride ($p < 0.001$). Respondents' category of water fluoride level was not significantly associated with tap water intake ($p = 0.122$).

Conclusion: The majority of subjects agreed that fluoride was beneficial and the level of fluoride in the water was not related

to tap water intake. However, additional research is needed about the complex relationships among knowledge, attitudes, and behaviors concerning fluoride and their associations with tap water intake.

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

66. Knowledge, Attitudes, and Behaviors Concerning Topical and Systemic Fluoride



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Objective: To inform future efforts to improve oral health education regarding systemic and topical fluoride, it is important to assess the knowledge, attitudes, and behaviors of adults surrounding this topic. The objective of this

study is to investigate the knowledge, attitudes, and behaviors toward fluoride in two rural Iowa communities, Raymond and Gilbertville.

Methods: A 20-question Qualtrics survey was formulated to target adults, aged 18+, residing in optimally fluoridated Raymond (0.70ppm) and suboptimally fluoridated Gilbertville (0.35ppm), Iowa. Questions assessed knowledge, attitudes, and behaviors toward water fluoridation and topical fluoride. Descriptive and bivariate (chi-square and Kendall's tau) analyses were conducted. Associations between demographic factors and knowledge, attitudes, and behavior questions were assessed.

Results: Ninety-six subjects completed the questionnaire. 54.2% reported not knowing if their tap water contained fluoride and 76.0% if their water contained the CDC-recommended fluoride concentration. 18.8% said that having fluoride in their tap water would increase their chance of drinking it. 60.4% reported they prefer toothpaste as their primary source of fluoride. Respondents from Raymond were more likely, but not significantly ($p = 0.123$), to say fluoridated tap water would increase their chance of drinking it (21.1% vs. 15.8%).

Conclusion: Although the majority of respondents were in favor of systemic and topical fluoride, there is ongoing need for education. Being able to identify gaps in knowledge, attitudes, and behaviors toward fluoride can be useful to dentists and public health professionals when educating the public on preventive treatments and the benefits of fluoride.

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

67. Factors Influencing Orthodontic Treatment Time and Completion



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Objective: To assess the influence of patient demographics, appointment attendance, oral hygiene, case complexity, and community factors on orthodontic treatment length and completion among children and adolescents.

Methods: The data source was electronic dental records of patients aged 8-18 that initiated treatment at the University of Iowa College of Dentistry, Orthodontic Residency Clinic between 2009 and 2019. Variables included patient demographics (age, biological sex, and payment method), community factors (distance from home address to clinic, urbanicity), appointment attendance (failed/late-cancelled appointments), oral hygiene, and case complexity (Angle's Classification Type and Salzmann Score). Bivariate and multivariate statistical analysis were performed.

Results: The study population included 1,504 patients (658 male, 846 female). The mean age at treatment initiation was 13.7 years. A total of 79.3% (n=1192) patients completed treatment within the study timeframe. In multivariate analyses, treatment completion was associated with driving distance (0.002), cancelled appointments frequency (<0.001), failed appointment frequency (<0.001), initial Salzmann Score (0.019), Angle's Classification Type (0.014) and oral hygiene notes (<0.001). The average treatment time was 24.2 months, and factors associated with treatment time included age at treatment initiation (<0.001), cancelled appointment frequency (<0.001), failed appointment frequency (0.004) and urbanicity (0.030).

Conclusion: The results suggest that a multitude of variables, including distance, appointment attendance, age, payment method, case difficulty, and patient compliance may influence treatment length and completion. This study may provide insight into individual, social, community, and case complexity factors that may be barriers to patients completing treatment, and help practitioners make treatment plans that consider these barriers.

Supported by: Iowa College of Dentistry Summer Research Funding; University of Iowa College of Dentistry Student Research Program.

68. University of Iowa Dental Student Debt and Career Path Implications



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Objective: The Resident Education Deferred Interest Act was introduced into the US Congress this year 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, on 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 will be administered to all currently enrolled dental students at the University of Iowa using the Qualtrics software. The survey instrument will ask students about their current post-dental school career interests, any research experience that they have had, their current financial status, and demographic information. The financial status questions will specifically ask students about the types of loans that they have received to pay for dental school and the interest rates associated with those loans.

Conclusion: Results of this study could be used to 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 certain career choices, particularly residency programs.

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

69. Smile Enhancement Using a Gingival Mask Prosthesis: A Case Report



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Objective: Alveolar bone loss resulting from periodontal disease or its therapy can lead to esthetic and functional complications, including formation of "black triangles" and elongated clinical crowns. Treatment options include surgical interventions and/or prosthetic management. Gingival replacement prostheses may be fixed or removable and may be made from acrylics, silicones, porcelain materials, or composite resins. Gingival prostheses have been proposed as a conservative and cost-effective solution. This case report describes the use of an ethylene propylene copolymer removable gingival prosthesis to improve esthetics in an area of advanced tissue loss.

Methods: A 45-year-old female with Kennedy Class 3 partial edentulism was referred to prosthodontics for a removable partial denture (RPD) 3 years after osseous surgery. After a thorough patient interview, the RPD treatment plan was expanded to include a gingival prosthesis to address her continued esthetic concerns. First, an intra-oral optical scan was performed, and a model printed. Then a gingival wax-up, putty matrix, and soft silicone mask prototype were fabricated. Once the form of the prototype was approved by the patient, a dental laboratory duplicated the printed model and fabricated the definitive prosthesis using the injection method with Duraflex™ material. OHIP-14 and PIDAQ surveys were completed by the patient 9 months post-treatment to assess oral health-related quality of life (OHRQoL).

Results: The patient was pleased with the prosthesis overall. We present pre-treatment and post-treatment clinical photographs for comparison.

Conclusion: The purpose of this report was to explore the effectiveness of a less-common prosthetic treatment for advanced tissue loss. The results illustrate a successful outcome where a gingival prosthesis improved esthetics at an affordable cost to the patient. To measure the effectiveness of gingival prostheses more reliably, further studies could compare OHRQoL outcome measures between this gingival prosthesis and other surgical or prosthetic interventions.

70. Investigating the Association Between Insurance Type and Pediatric Referral Patterns



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Objective: The objective of this study was to investigate characteristics of patients referred to the University of Iowa Pediatric Dental Clinic (UIPDC) based on type of managed care insurance. Findings obtained from this study will provide a better understanding of characteristics of managed care participants being referred to the UIPDC.

Methods: Data were previously collected from a cohort of 1,611 participants referred to the UIPDC between July 1, 2020 and December 31, 2021. This study focused on participants referred to the UIPDC between July 1, 2021 and December 31, 2021, following the transition of participants' Medicaid coverage to managed care. Only participants enrolled in the managed care program were included. Referral characteristics of 395 participants with either Plan A or Plan B insurance were investigated. Participant characteristics included: age, gender, duration between initial appointment and treatment completion, distance from participant's hometown to the UIPDC, size of the patient's hometown, referring provider, and treatment needs of the patient. Descriptive statistics were computed, and bivariate analyses were performed (alpha=0.05).

Results: The size of the patient's hometown (p=0.019) and type of referring provider (p<0.001) were significantly associated with insurance plan. Plan A participants were more

likely to live in a community with a population of 25,001-75,000 (30.2% vs. 18.3%), while Plan B participants were more likely to live in a community with a population of more than 75,000 (33.9% vs. 22.8%). Participants with Plan A insurance were more likely to be referred by a specialist (31.6% vs. 17.2%), while Plan B participants were more likely to be referred by a general dentist (82.8% vs. 67.5%).

Conclusion: Participants with Plan A are more likely to live in a smaller community and be referred by a specialist, while those with Plan B are more likely to live in a larger community and be referred by a general dentist.

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

71. Conceptualizing Next Patient Interaction: Differences From Ideal/Desired Patient Outcomes



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Objective: Conceptualizing the next patient interaction is logical, essential, and largely done intuitively; however, limited literature is available.

Purpose: To elicit student reflections on four questions relating to differentiation from the ideal and desired patient interaction outcomes. Secondary purposes are to classify responses for respective questions and to review faculty assessments of student responses.

Methods: The exercise was introduced in the first clinical prosthodontics experience after simulation clinic course and was completed in the fall of 2023. Following a published pilot study, four open-ended questions were selected on 1) differentiation from the ideal, 2) desired outcome(s), 3) capabilities, and 4) consequences/prognosis. A total, forty-two students provided responses to the four questions. Nine different faculties assessed the exercise.

Results: 100% of students provided responses to all four questions. No overlap in responses was observed among the four questions. The average of student's number of words per response was 16 to 20. Among faculty assessment, 100% applying the step, and 83% judging the student to have grasped the concept in the question. Authors categorized responses into natural categories for each question. Subsequently, authors separately assigned responses to categories. The agreement rate was 90%. No overlap in responses was observed among the four questions. Students progressed from gathering tangible and intangible information in Question #1 to proactively guiding the patient in Questions 2, 3, and 4—a progression from empathy to compassion. Students rated the experience as practical.

Conclusion: The exercise elicited different kinds of reflection on four topics encountered by the general dentist at each patient interaction. The format was succinct with acceptance by students and faculty.

72. Evaluating the Accuracy of Intraoral Scanner Post Space Impressions



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Objective: There are currently few clinical guidelines for digital post space impression techniques. The accuracy of intraoral scanners (IOS) for post space impressions is an area of ongoing study as evidence is lacking for IOS performance with different tooth types and post space lengths. The purpose of this in-vitro study was to evaluate the trueness of digital post space impressions by 2 different IOS, for 3 different tooth types and 4 post space lengths.

Methods: 3 different maxillary tooth types (central incisor, first premolar and first molar) with 4 different post space lengths (10mm, 11mm, 12mm, and 13mm) were digitally designed and 3D-printed. 9 digital post space impressions were made for each sample using intraoral scanners TRIOS4 and Primescan. Each scanned post space was measured and compared with the reference file using CAD software for trueness and % volume difference. One-way Welch ANOVA and pairwise Welch t-tests were used to analyze normally distributed data. Kruskal-Wallis and post-hoc Dunn tests with Holm correction were used to analyze non-normally distributed data, and a permutation test with post-hoc analysis for multiple tied values. The significance level was set at P<.05.

Results: Statistically significant differences exist among the post space length groups for both RMS and volume capture within each tooth type (P<.05). For trueness, median RMS ranges from 26µm to 134µm with central incisor having the highest trueness, then molar, followed by premolar. Volume capture mean percent range from 76.3% to 100%, with the premolar group having lower volume results, then central followed by molars.

Conclusion: TRIOS4 intraoral scanner fails to capture the apical 2mm of any post space in this study. Primescan showed high trueness and volume capture of post space digital impression for maxillary central incisors and molars up to 13mm length and maxillary first premolar up to 12mm.

Supported by: University of Iowa College of Dentistry Clinical/ Dental Education Research Initiative Support Program (CRISP).

73. Meta-Analysis of Metatranscriptomic Profiles in Oral Microbiome Linked to Periodontitis



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Objective: Periodontal diseases are a significant global public health concern. Recent research emphasizes the role of oral microbes in periodontitis. Utilizing metatranscriptomics, our study conducts a meta-analysis to unveil microbial influences on periodontitis, offering valuable insights into gene expression dynamics associated with oral health.

Methods: In our pursuit to comprehend alterations in the oral metatranscriptome associated with periodontal diseases, we conducted a systematic review of contemporary literature. Following this, we performed a rigorous re-analysis of publicly accessible metatranscriptomic datasets to ensure a uniform assessment. The outcomes of our study highlight distinct shifts in microbial attributes, spanning both species and functions, particularly evident when comparing periodontitis to healthy controls.

Results: Our comprehensive review of periodontal metatranscriptomic studies was conducted between 2016 and 2023. Collectively, these investigations underscore a substantial link between periodontal diseases and oral microbial imbalances, characterized by a significant reduction in microbial diversity and heightened activities in pathogenic functional categories. Notably, specific bacterial strains exhibit varying alterations across studies, while functional attributes associated with periodontal diseases demonstrate a higher degree of consistency compared to taxonomic composition. Key insights from our meta-analysis reveal prevalent occurrences of *Tannerella* sp. and *Actinomyces* sp. in periodontitis, the pivotal role of Cysteine peptidases in the chronic form, and the substantial impact of carbohydrate metabolism and amino acid biosynthesis pathways on overall oral health.

Conclusion: Rapidly advancing, oral metatranscriptomics holds promise in decoding host-microbe interactions specific to periodontitis. It pioneers targeted therapeutic approaches by examining the impact of external factors on oral health within this context, deepening our understanding of mechanisms. With bioinformatics as its foundation, metatranscriptomics revolutionizes our insights into the molecular intricacies of periodontitis, shaping innovative strategies for diagnosis and treatment.

74. Oral Health of Nursing Facility Older Adult Residents Utilizing Teledentistry



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Objective: Older adults living in nursing homes experience a myriad of barriers to oral care.

Programs that utilize teledentistry to diagnose oral disease and treatment plan care for older adults need to take into consideration the disease burden and needs of the population served. This study examined the oral health status of adults in two nursing homes in Iowa that were enrolled in a program that utilized asynchronous teledentistry to improve access to dental care.

Methods: Research exams were completed on older adults enrolled in the program. Data were obtained on residents' demographics, general and oral health status, cooperation, mobility, and urgency of treatment needs. Descriptive results of residents' oral disease burden are presented.

Results: A total of 34 residents were examined, among whom 32% had soft tissue lesions, most were dentate (74%; mean of 14 remaining teeth), and 21% required urgent dental treatment.

Among dentate residents (n=25), approximately two-thirds (64%) had severe gingival inflammation. All residents had caries experience (DMFT>=1), with caries experience severity (mean DMFT) of 19 (range: 5 - 28), 92% had untreated caries, [mean DT: 5.3 (range 0 - 15)], and 40% had arrested caries (mean: 0.6 (range: 0 - 3)).

Conclusion: Results revealed high oral disease burden indicative of unmet dental need among older adults in participating nursing homes. The high disease burden underscores the need for programs like teledentistry models that extend the reach of dental clinics to provide diagnostic and preventive care and improve dental care access for older adults.

Supported by: Delta Dental of Iowa Foundation.

75. Oral Microbiome Feature-Based Machine Learning Approach to Predicting Late-Life Depression



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Objective: Besides genetics and environmental factors, accumulating evidences supports that the poor oral health or alterations in oral microbiome is closely related to depression. Given the high dimensional nature of the oral microenvironment, we utilized machine learning (ML) combined with microbiota and inflammatory factors at the salivary level to identify depression among the elderly.

Methods: This study examines the structure and composition of the salivary microbiome and salivary level of pro-inflammatory cytokines (IL-1β, TNF-α and IL-6), human cathepsin B (CTSB), and cortisol in elderly who suffered from late-life depression (n=64) and matched controls (n=74) using 16S ribosomal RNA sequencing and enzyme-linked Immunosorbent assay (ELISA) analysis. Depression was accessed using the geriatric depression scale (GDS). Four supervised ML algorithms were trained to classify samples including random forest (RF), support vector machine (SVM), decision tree and extreme gradient boosting (XGBoost). To ensure the development of a robust and reproducible model, we reduced the data dimensionality by Pearson correlation coefficient and applied model averaging across a set of 100 models from repeated application with ten-fold cross-validation on training set. In addition, personalized depression biomarker contributions were computed using feature importance of RF and Shapley additive explanations (SHAP).

Results: A total of over 2,000 features, including genus, species and pro-inflammatory factors, were analyzed using the Pearson correlation coefficient method. Though a cyclic iterative approach, the optimal correlation coefficient was determined be approximately 0.18, resulting in the selection of 180 features. The RF model with the best-performance as measured by the receiver operating characteristic curve (AUC) are 0.84 and an accuracy of 86%.

Conclusion: Certain oral pathogens have the strong power of prediction on late-life depression. This study adds new evidence to the cross-talk between oral health and mental health, which may facilitate non-invasive health screening methods for the elderly.

76. MiR-223-3p Regulation of Cementoblast Apoptosis Under Hypoxia Via FoxO3 Targeting



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Objective: Cementoblast apoptosis in hypoxic conditions may contribute to root resorption during orthodontic tooth movement. Our previous sequencing data revealed a significant decrease in miR-223-3p expression and an increase in FoxO3 expression in cementoblasts under hypoxia. Both miR-223-3p and FoxO3 are known to play crucial roles in apoptosis and inflammation regulation. This study aims to investigate their roles and potential mechanisms in cementoblast apoptosis under hypoxic conditions.

Methods: The miR-223-3p mimics and inhibitors were introduced into OCCM-30 immortalized odontoblasts to allow modulation of miR-223-3p expression. Additionally, FoxO3 overexpression plasmids and small interfering RNAs were employed for the manipulation of FoxO3 levels. The expression of apoptosis-related genes and the mitochondrial DNA damage-related genes, including Caspase-3, Bax, Bcl-2, and mtDNA, was quantified using real-time quantitative PCR (RT-PCR). The protein levels of Cleaved-caspase-3, Bax, Bcl-2, and Mfn2 were assessed through Western blot analysis. Intracellular ROS, mitochondrial ROS, and mitochondrial membrane potential (MMP) levels were determined via fluorescent staining.

Results: Hypoxic conditions led to increased cementoblast apoptosis, accompanied by oxidative stress and mitochondrial dysfunction. MiR-223-3p exhibited an anti-apoptotic effect by targeting FoxO3. Overexpression of FoxO3 reversed the anti-apoptotic effect of miR-223-3p, and the ROS inhibitor NAC mitigated FoxO3's detrimental impact on cells.

Conclusion: In a hypoxic environment, miR-223-3p reduces cell apoptosis by targeting FoxO3, while FoxO3 may enhance cell apoptosis by promoting mitochondrial dysfunction and oxidative damage.

77. Evaluation of Root and Crown Movement via Palatal Superimposition



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Objective: Superimposition of digital models based on palatal region with roots segmented automatically from CBCT before treatment could be used to evaluate root and crown movement during orthodontic treatment. This study aimed to evaluate the precision and reliability of palatal superimposition in evaluating root and crown movement compared with CBCT maxillary voxel-based superimposition.

Methods: The sample consisted 16 patients whose oral scans and cone-beam computed tomography (CBCT) were obtained before and after treatment. Palatal superimposition with roots between oral scans before and after treatment was performed to measure the crown and root movement of upper first molars (U6s) and upper centra incisors (U1s). As a control, CBCT before and after treatment were superimposed using voxel-based methods from Dolphin Imaging to measure the crown and root movement of U6s and U1s. The crown and apical root movement distance of these two methods were compared in the three-dimensional direction.

Results: Data distribution was normal by the Shapiro-Wilk W test. The paired t test was used to compare the crown and root movement distance between CBCT maxillary voxel-based superimposition and palatal superimposition. For crown movement distance, there are no significant difference between the two methods (P > 0.05). As for root movement distance, there are also no significant difference between the two methods except for the right maxillary molars (P < 0.05). Intraclass correlations of these two methods were larger than 0.8, which showed great reliability.

Conclusion: The palatal superimposition showed similar results to the CBCT maxillary voxel-based superimposition. With roots segmented automatically from CBCT before treatment, palatal superimposition was a precise and reliable method to evaluate crown and root movement during treatment.

78. Multifunctional Coating Against Oxidative Stress for Peri-Implant Soft Tissue Regeneration



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Objective: Since the microgap between implant and surrounding connective tissue creates the pass for pathogen invasion, sustained pathological stimuli can accelerate

macrophage-mediated inflammation, therefore affecting peri-implant tissue regeneration and aggravate peri-implantitis. As the transmucosal component of implant, the abutment therefore needs to be biofunctionalized to repair the gingival barrier.

Methods: We developed and characterized a mussel-bioinspired implant abutment coating containing tannic acid (TA), cerium and minocycline (TA-Ce-Mino). Then the drug delivery behavior, antibacterial function and immunoregulation capacity of TA-Ce-Mino were evaluated. Besides, angiogenesis effect of human umbilical vein endothelial cells (HUVECs) under inflammatory environment and effect of ta-ce-mino on attachment promotion and activation of human gingival fibroblasts (HGFs) were further tested *in vitro*. Finally, we tested its multifunctional effect *in vivo* study.

Results: TA provides pyrogallol and catechol groups to promote cell adherence. Besides, Ce3+/Ce4+ conversion exhibits enzyme-mimetic activity to remove reactive oxygen species while generating O₂, therefore promoting anti-inflammatory M2 macrophage polarization to help create a regenerative environment. Minocycline is involved on the TA surface to create local drug storage for responsive antibiosis. Moreover, the underlying therapeutic mechanism is revealed whereby the coating exhibits exogenous antioxidation from the inherent properties of Ce and TA and endogenous antioxidation through mitochondrial homeostasis maintenance and antioxidases promotion. In addition, it stimulates integrin to activate PI3K/Akt and RhoA/ROCK pathways to enhance VEGF-mediated angiogenesis and tissue regeneration.

Conclusion: Aiming to the clinical status as the lack of biofunctionalization on implant abutment, we have constructed a TA-Ce-Mino coating on PEEK and Ti substrates for abutments for peri-implantitis treatment and prevention. A multifunctional strategy was verified like the pH responsive and long-term antibacterial activity, ROS scavenge ability, M2 polarization, microenvironment regeneration and boosted angiogenesis. To sum up, our study had provided a critical insight into the design of host defensive system repair containing connective tissue barrier and immunoregulation.

79. Electroacupuncture Alleviates Alveolar Bone Loss in Experimental Ligature-Induced Periodontitis



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Objective: Electroacupuncture (EA) regulates distant body physiology through somatic sensory autonomic reflexes, balances the microbiome, and can promote the release of immune cells into bloodstream, thereby inhibiting severe systemic inflammation. This makes it possible to use EA as an integrated treatment for periodontitis.

Methods: In this study, EA was applied to the ST36 acupoints in a ligature-induced periodontitis (LIP) mouse model. Then the effects of EA on periodontal myeloid cells, cytokines, and the microbiome were comprehensively analysed using flow

cytometry, quantitative Polymerase Chain Reaction (PCR), and 16 S sequencing.

Results: Results demonstrated that EA could significantly relieve periodontal bone resorption. EA also suppressed the infiltration of macrophages and neutrophils, reduced gene expression of the pro-inflammatory cytokines IL-1 β , IL-6, IL-17 and TNF- α , and increased expression of the anti-inflammatory factors IL-4 and IL-10 in periodontal tissues. Moreover, composition of the periodontal microbiome was regulated by EA, finding that complex of microbiota, including supragingival *Veillonella*, subgingival *Streptococcus*, and subgingival *Erysipelatoclostridium*, were significantly reduced. Meanwhile, nitrate and nitrate-related activities of subgingival microbiota were reversed. Network analysis revealed close relationships among *Veillonella*, *Streptococcus*, and *Bacteroides*.

Conclusion: Our study indicates that EA can effectively alleviate inflammation and bone resorption in LIP mice, potentially via the regulation of myeloid cells, cytokines, and periodontal microbiome.

80. Bond Strength of Intraradicular Posts Using Different Cementation Protocols



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Objective: This study aimed to evaluate the influence of a late activation protocol of two dual-cured resin cements with different photoinitiators on the bond strength of fiber-glass posts to the different thirds of intraradicular dentin using two light-curing devices.

Methods: Single-rooted premolars (n=130) had their clinical crowns removed 1mm above the cemento-enamel junction and received an endodontic treatment. After that, the teeth were randomly divided into ten experimental groups (n=13/group) according to the dual-cured resin cements (Multilink N or Variolink Esthetic DC), late activation protocol (chemical cure, immediate light-curing, and light-curing after 5 minutes), and light-curing device (Elipar DeepCure-S and VALO) used for the luting procedure of anatomized posts to intraradicular dentin. For bond strength evaluation, samples were sectioned perpendicularly to the long axis of the tooth, and one slice was obtained from each third of the root canal (cervical, middle, and apical). The sections were subjected to a push-out test in a universal testing machine. The mean bond strength (MPa) values were submitted to 3-way ANOVA and Tukey's post-hoc tests ($\alpha=0.05$).

Results: Higher bond strength values were obtained for cervical and medium thirds than for the apical third. The apical third presented the lowest bond strength values, even for the chemical activation groups, with a statistically significant difference for the cervical and middle thirds ($p<0.05$). There was no statistically significant difference ($p>0.05$) in bond strength when comparing the cements as well as among the different activation protocols.

Conclusion: Thus, different light-curing devices, activation protocols, and the presence of different photoinitiators in dual-cured resin cements did not influence the bond strength of glass-fiber post to intraradicular dentin.

Supported by: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

81. Oral Squamous Carcinoma Cell Reprogramming TAM via Intercellular Mitochondria Transfer



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Objective: To investigate the underlying mechanisms of the alterations in the phenotype and function of tumor-associated macrophages (TAMs) in the tumor microenvironment (TME) of oral squamous cell carcinoma (OSCC).

Methods: We collected samples from OSCC patients and established subcutaneous xenografts in nude mice. Then, we co-cultured OSCC cancer cells with THP-1 derived macrophages (M ϕ) *in vitro* to simulate the OSCC TME. Western blotting (WB), qRT-PCR, immunohistochemistry (IHC), immunofluorescence (IF), and flow cytometry (FACS) were performed to evaluate the alterations of TAMs in the OSCC TME. RNA-seq, Seahorse assays, mass spectrometry-based metabolomics analysis, enzyme-linked immunosorbent assay (ELISA), flow cytometry (FACS), and scanning electron microscopy were carried out to uncover the underlying mechanisms of the alterations.

Results: Macrophages in the OSCC TME showed a mixed TAM phenotype with a predominance of immunosuppression. Cancer cells enhance intracellular lipid metabolism is involved in regulating macrophage M2-like TAMs polarization. Further investigations revealed that the enhanced lipid metabolism in TAMs is associated with TNT-mediated mitochondrial transfer between cancer cells and TAMs. By inhibiting the formation of TNTs between cancer cells and TAMs, we can modify the metabolic behavior of TAMs and alter their characteristics. The combination of TNT inhibitors with α -PD1 antibodies effectively suppresses the immune-suppressive characteristics of TAMs within the tumor and enhances the therapeutic effectiveness *in vivo*.

Conclusion: The transfer of mitochondria from OSCC cancer cells to TAMs via TNT is an important way to regulate macrophages in OSCC TME. Therefore, we posit that inhibiting the transfer of mitochondria from OSCC cancer cells to TAMs through TNT may be a promising therapeutic approach for treating oral squamous cell carcinoma

82. Exosomes Pretreated With Metformin Promote Gingival Wound Healing in Diabetes



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Objective: To evaluate the wound healing potential of exosomes pretreated with metformin in the mouse model with type 2 diabetes mellitus (T2DM).

Background: Diabetic wound, characterized by slow and incomplete healing, remains a clinical challenge among individuals with diabetes. Exosomes, the extracellular membrane-bound vesicles, can influence cellular behavior and play an essential role in cell-to-cell communication. In therapeutic practice, exosomes are prone to be pretreated with drugs to enhance their function. However, the effect of optimized exosomes on diabetic wound healing is rarely reported.

Methods: *In vitro* experiment, we evaluated the effects of the exosomes pretreated with metformin in the wound healing process. The analyses of cellular proliferation, cellular migration, as well as angiogenesis were conducted. *In vivo* experiment, we constructed a microneedle-protein nanoparticles (PN) fiber membrane to realize the controlled release of exosomes. The mice were assigned into five groups: sham group, T2DM group, Gelma-PN group, Exo-PN group, and metformin exosomes (Met-exo) group. The histological detections were performed to evaluate the wound healing status.

Results: The results showed that the bio-materials could restore H2S homeostasis in diabetic conditions, and its function in accelerating the wound healing process was also confirmed in mouse model with T2DM. Mechanistically, we demonstrated that Met-exo could upregulate the level of hydrogen sulfide (H2S) in fibroblasts via activating cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE).

Conclusion: In summary, we show that pretreated with metformin, the exosomes derived from PDLSCs can serve as a positive regulator for diabetic wound healing. Furthermore, the potential therapeutic applications of Met-exo in diabetic wound healing is demonstrated, considering its positive regulatory effects on H2S homeostasis.

83. Automatic Root Resorption Analysis in CBCT Using Deep Learning Methods



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Objective: Automatic three-dimensional dental model generation from CBCT can improve diagnosis and treatment planning efficiency and accuracy. However, the unclear boundaries between tooth and alveolar bone in CBCT scans can significantly influence the accuracy of

segmentation results. To achieve precise tooth segmentation, especially in the root region. We proposed the 3D-Unet method to accurately and quickly segment teeth from CBCT images. An automated 3D quantitative analysis of tooth morphology was conducted to investigate external root resorption after orthodontic treatment.

Methods: For extraction by an effective localization module, an integrated tooth segmentation model based on a combination of multi-scale semantic information with explicit anatomical connection and tooth bounding box is proposed. In this model, teeth are effectively segmented into their respective categories. Afterward, a 3D stereo model is created to automatically calculate tooth lengths, volumes, and surface areas. By integrating output data before and after orthodontic treatment, a root resorption classification is obtained. 300 pairs of CBCT from patients finishing orthodontic treatment were collected as training datasets. To validate the precision of the automatic morphology analysis method, a three-dimensional analysis of 30 patients was conducted manually as the golden standard.

Results: This segmentation model achieved high levels of accuracy and robustness despite difficult cases such as crowded teeth, radiographic artifacts, and missing teeth. The intra-class correlation coefficient (ICC) was found to be 1.000, 0.985, and 1.000 with an average error of 0.000 mm3, 0.024 mm, and 0.037 mm2 for assessing the volume, length, and surface area of root resorption, respectively.

Conclusion: The three-dimensional structure of teeth is extracted and automatic root resorption classification is performed in this system. Based on the results of the experiment, it can be concluded that the system provides a valuable tool for the analysis of external root resorption derived from CBCT. It will assist dentists in selecting the most appropriate treatment strategy to limit root resorption.

84. Type 2 Diabetes Aggravates Periodontitis Through Gut Microbiota



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Objective: This study aimed to investigate whether type 2 diabetes mellitus (T2DM) can aggravate periodontitis through gut microbiota.

Methods: In the first part, mice were divided into 4 groups: DM+Lig, DM+N, N+Lig, N+N. T2DM models were established in mice in DM groups. In the second part, mice were divided into 4 groups: FD+Lig, FD+N, FN+Lig, FN+N. Mice in FD groups received fecal microbiota transplantation (FMT) from T2DM donor mice while mice in FN groups received FMT from normal donor mice. In the third part, mice were divided into 4 groups: DFD+Lig, DFD+N, DFN+Lig, DFN+N. T2DM models were established in all the mice. Mice in DFD groups received FMT from T2DM donor mice while mice in DFN g

Results: Gut dysbiosis was observed in T2DM mice while mice with both T2DM and periodontitis showed more bone resorption compared with mice only with periodontitis. FMT from T2DM donor mice also aggravated periodontitis in mice compared with FMT from normal mice. On the contrary, when T2DM mice with periodontitis received FMT from normal mice,

the disordered gut microbiota was partly restored and alveolar bone resorption was reduced.

Conclusion: T2DM might aggravate periodontitis through disordered gut microbiota. Gut microbiota can be a potential therapeutic target for patients with both T2DM and periodontitis.

85. Low-Temperature Printed-Scaffold With Inorganic Bioglass Ink for Critical Bone Regeneration



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Objective: Additive manufacture is a promising strategy for bone regeneration of maxillofacial critical bone defects, while the difficulty to remove the organic additive and the hyperthermal post-printing sintering may impair its shape-fidelity and bioactivity. This study aims to construct a novel additive-free and low-temperature sintered 3D-printing ink and to validate its osteogenic capacity.

Methods: A novel additive-free inorganic ink for ceramics 3D printing was developed via electrostatic assembly of oppositely charged silica-based nanospheres. The synthesis was characterized by SEM and CLSM. The printability and mechanical properties were evaluated through rheological measurement and universal mechanics testing. Biocompatibility and osteogenic activity *in vitro* were evaluated using MC3T3-E1. Finally, rabbit calvarial critical bone defect model was used to verify the *in vivo* bone regeneration effect.

Results: The inorganic ink was successfully constructed with shear-thinning, self-healing behavior, and high elasticity. The fidelity, mechanical strength and bioactivity and osteogenic capacity *in vitro* were confirmed. Micro-CT and VG staining showed improved bone regeneration *in vivo*. Especially, contact osteogenesis induced by BG was observed via sequenced fluoresce and shape fidelity of newly formed bone for individualized defect was validated by histo-morphological evaluation, suggesting an accelerated and predictable bone regeneration. Biomechanically mediated CaMKII/ CREB signaling pathway was further revealed as underlying osteogenic mechanism using single-cell sequencing.

Conclusion: The proposed additive-free and low-temperature sintered novel inorganic ink is proved with outstanding printability, biocompatibility, and osteogenic activity, offering an efficient strategy for 3D-printing of bioceramic bone substitutes in maxillofacial bone regeneration.

86. Genetic-Epigenetic Interactions (meQTLs) in Orofacial Clefts Etiology



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Objective: Nonsyndromic orofacial clefts (OFCs) are the most common human craniofacial birth defects. Genome-wide studies have identified over 50 OFC risk loci; however, they account for a minority of the estimated risk, and many of them reside in non-protein coding regions with unknown function so far. Environmental and genetic risk factors can affect disease status by modulating gene expression via epigenetic processes, such as DNA methylation (DNAm). We hypothesize that aberrant DNAm and the resulting alterations in gene expression play an important role in the etiology of OFCs. Our goal is to determine whether cleft-associated SNPs influence DNAm levels at target CpG sites (meQTLs), contributing to increased OFC risk.

Methods: Based on preliminary data suggesting associations between cleft GWAS SNPs and genomic DNA methylation, we selected 13 SNP-CpG associations (meQTLs) for replication in an independent cohort of 362 same-sex pairs of cleft-discordant siblings (DNA from blood and saliva). After bisulfite conversion, we used methylation-specific qPCR (Qiagen EpiTect™ MethyLight) to measure methylation levels of each CpG site. We then combined genetic and methylation data for an interaction analysis of each SNP-CpG pair using the R package MatrixeQTL in a linear model. Blood and saliva samples were analyzed separately and combined.

Results: We replicated 6 SNP-CpG associations. The methylation quantitative trait loci (meQTL) analysis showed interaction between rs13041247(MAFB)-cg18347630(PLCG1) (p=0.04-blood); rs227731(NOG)-cg08592707(PPME1) (p=0.02-all); rs227731(NOG)-cg10303698(CUEDC1) (p=0.002-all); rs987525(8q24)-cg16561172(CASC11) (p= 5.41e-07-saliva, p= 1.33e-06-all); rs7590268(THADA)-cg06873343 (p=0.03-blood); rs7078160(VAX1)-cg09487139 (p=0.04-blood).

Conclusion: Our results confirm that some of the common noncoding variants detected through GWAS studies can influence the risk of OFC via epigenetic mechanisms, such as DNA methylation, which can ultimately affect and regulate gene expression. Given the large prevalence of non-coding SNPs in most OFC genome wide association studies, our findings can potentially address major knowledge gaps, like missing heritability, reduced penetrance, and variable expressivity associated with OFC phenotypes.

Supported by: NIDCR K01 DE027995, R37 DE008559, U01 DE020057, R01 DE012472, R21 DE016930, R01 DE014667, R01 DE028300.

87. Different Stimulation Strategies to Study the MMPs Expression by DPSC



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Objective: To explore novel model systems for studying dentin-pulp complex response to caries progression at the molecular level under *in vitro* conditions. We evaluated the expression of matrix metalloproteinases (MMP) -2 and -3 by dental pulp stem cells (DPSC) treated with different stimulants such as bacteria that have been shown to play a role in caries development including viable cells and/or their metabolites.

Methods: *Streptococcus mutans* (SM) and *Lactobacillus rhamnosus* (LR) were plated on blood agar and transferred to Dulbecco's modified eagle medium (D-MEM) containing 2% FBS and 1% MEM-vitamin. After culturing for 24 h, bacterial cells and filter sterilized supernatant (bacterial filtrates) were used for DPSCs stimulation. Lipopolysaccharide (LPS) from *Escherichia coli* and lipoteichoic acid (LTA) from *Bacillus subtilis* were used as control bacterial components. DPSC were treated with SM or LR at multiplicities of infection (MOI) 1:100 and 1:10 with/without 20% bacterial filtrates, 10µg/ml LPS, or 15µg/ml LTA for 24 h. Protein expression of MMP-2 and MMP-3 in the culture supernatant was estimated using ELISA. Linear mixed models were employed to determine significant differences in stimulation profiles (α=0.05).

Results: Stimulation of DPSC with SM or LR filtrates did not result in significant changes in the levels of MMP-2 (p>0.174) or MMP-3 (p>0.134). DPSC stimulated with bacterial cells or in combination with bacterial filtrates did not show significant variation in the expression levels of MMP-3, but most of the treatment groups had significant variation in the expression of MMP-2. The addition of LPS and LTA alone or along with bacterial cells significantly increased the levels of MMP-3 in comparison to control (p<0.05).

Conclusion: Bacterial co-culture or stimulation with filtrates did not result in an effective DPSC response in terms of variations of MMP3 expression in contrast to MMP2, and LPS/ LTA addition alone or with bacteria induced a valiant cellular response.

Supported by: NIDCR K08 DE029490.

88. Retrospective Analysis of Erosive Tooth Wear by Gender and Age



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Objective: This study assessed the likelihood of potential risk indicators for erosive tooth wear (ETW) according to gender and age groups, in a school clinical setting.

Methods: Data were retrieved from patient records in Axiom database from College of Dentistry-University of Iowa. A total of 2,217 adult patients were registered with ETW from 2019 to

2023. They were categorized by gender (male and female), and by age group (18-34, 35-54, and ≥55 years). Self-reported risk-indicators included gastroesophageal reflux disease (GERD), eating disorders (ED), dry mouth (DM), sleep apnea (SA), tooth clenching/grinding/bruxism (CGB), orthodontic treatment (OT), alcohol consumption (AC), daily alcohol consumption (DAC), brushing teeth twice/day (B2x), and brushing teeth more than twice/day (B>2x). brushing teeth more than twice/day (B>2x). brushing teeth more than twice/day (B>2x). The difference in expression between risk indicators and gender, as well as between risk indicators and age groups, was analyzed using Fisher's exact test and logistic regression, respectively (α=0.05).

Results: For gender, statistically significant differences were observed. Males had lower odds of reporting ED (OR=0.081; p<0.0001), DM (OR=0.576; p<0.0001), CGB (OR=0.757; p=0.0096), OT (OR=0.663; p=0.0011), and B2x (OR=0.538; p<0.0001), and increased odds of reporting SA (OR=1.674; p=0.0096) and DAC (OR=1.950; p=0.0013). In age groups, the 35-54 group had greater odds of reporting GERD (OR=2.422; p<0.0001), DM (OR=2.986; p<0.0001), SA (OR=4.088; p=0.0003), AC (OR=1.318; p=0.019), DAC (OR=2.359; p=0.0057) and B>2x (OR=2.029; p<0.0057), and lower odds of reporting ED (OR=0.272; p=0.0057), CGB (OR=0.527; p<0.0001) and OT (OR=0.615; p=0.0011) compared to the 18-34 group. For ages ≥55, the odds of reporting GERD (OR=1.463; p=0.05), DM (OR=1.430; p=0.05), and SA (OR=2.545; p=0.05) were significantly higher than the 18-34 age group.

Conclusion: Most of significant differences in potential ETW risk indicators were in females and in the 35-54 age group. These findings might help health professionals in understanding the impact of different risk indicators in distinct patient groups.

Supported by: NIDCR T90 DE023520.

89. Enrollment Challenges Among Low-Income WIC-Enrolled Pregnant Mothers



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Objective: Studies working with low-income high-risk populations face many challenges in recruitment and enrollment. The purpose of this poster is to report on the progress thus far in the current NIDCRUH3 "Birth—Cavity Free" (BTCF) clinical trial.

Methods: The planned BTCF sample will include 634 pregnant women from 12 WIC clinics across Eastern Iowa whose future children will be followed until they are 36 months of age. Inclusion criteria are WIC participating pregnant women who are 18 to 45 years old; between 12 and 36 weeks of the gestational period; able to speak, understand and read English or Spanish; and no intention to move away in the next 4 years. Participant recruitment status is summarized.

Results: Of the 1326 WIC appointments for enrolled pregnant mothers which were targeted for recruitment, 746 (56%) of the mothers showed up for their appointments; 302 of them were either not interested in participation (n=160) or did not

fit the study inclusion criteria (n= 142) (e.g., past gestation window, underage, non-English and non-Spanish speaking). Out of the 746 who attended their appointments, 444 expressed interest in participating in the study (33.5% of total 1326 appointments, and 60% of the 746 who showed for their scheduled appointments). All of these were screened and had their information recorded into Microsoft Teams database. Currently, 144 have been enrolled in the study and 60 are waiting to reach the gestational window to be enrolled.

Conclusion: Recruiting from a low-income high-risk population has proven to be challenging. It is hoped that strategies to increase the gestational window from initially 24-32 weeks to now 16-36 weeks and adding the Johnson County WIC sites will improve enrollment numbers.

Supported by: NIDCR UH3 DE029443.

90. Medicaid Dental Insurance Coverage for Undocumented Immigrant Population



Khlood.S. Saeed¹, S. Iribarren¹, J. C. Reynolds¹

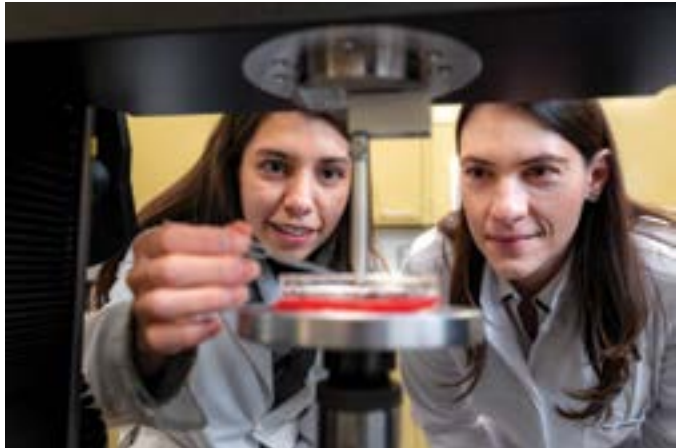
¹University of Iowa, Iowa City, IA

Objective: The aim of this environmental scan was to examine state policies regarding Medicaid medical and dental eligibility and coverage for undocumented-immigrant populations with low income. This scan is important to understand the landscape of access to dental coverage for a population that has numerous barriers to getting needed care.

Methods: A grey literature search was conducted to identify Medicaid coverage and policies for three groups of undocumented immigrants: children, pregnant individuals, and non-pregnant adults. Information was collected from state Medicaid websites, reports from national health policy research organizations, and other sources. Eligibility for medical coverage was categorized as full, limited, or none. In full Medicaid states, the search focused on the degree of Medicaid medical and dental coverage for the three population groups.

Results: In four states, non-pregnant adults were eligible for full Medicaid coverage, all of which included extensive dental coverage. For children, 11 states provided full Medicaid coverage, including dental benefits. For pregnant individuals, nine states provided full Medicaid coverage, all of which included extensive dental coverage. The remaining states offered limited or no Medicaid coverage, with some providing coverage for only labor and delivery services and emergency care.

Conclusion: Overall, relatively few states provide access to full medical or dental coverage in Medicaid for undocumented immigrant populations with low income. These findings suggest that in most states, restricted access to Medicaid coverage likely remains a significant barrier to receipt of dental care and improved oral health outcomes among undocumented immigrant populations.

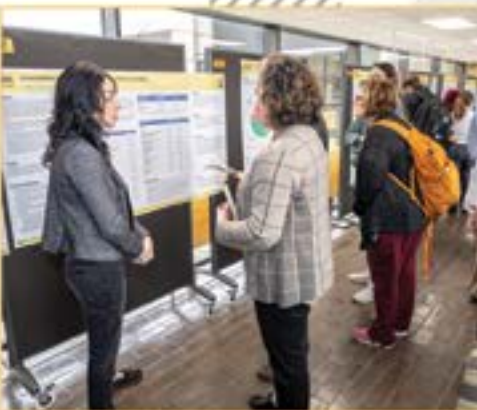
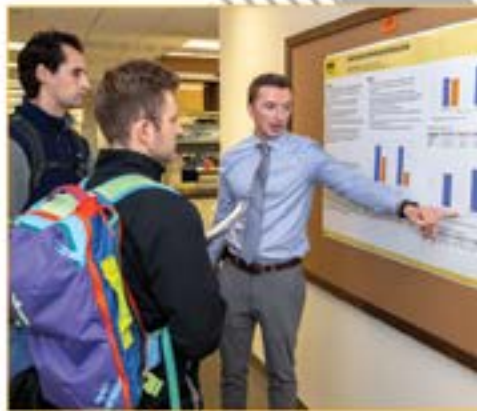
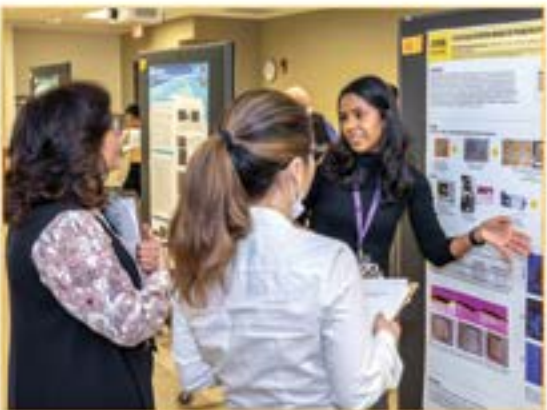


Cristina Vidal (right), assistant professor in the Department of Operative Dentistry, is a mentor for students in the Dental Student Research Program. Their research typically focuses on caries risk and the strength and stability of natural dentition, bonded materials, and other restorations. Mariana Queiroz is a visiting scholar and is mentored by Vidal.

3. Impact

- After graduating from dental school, most participants in the student research program enter private practice, either directly or after specialty training. For these students, learning about the research process that provides the foundation for clinical procedures and decision-making makes a profound difference in clinical practice, improving a student's ability to understand and process evidence-based advances in dental practice and helping them develop critical-thinking skills for practicing evidence-based dentistry.
- A small group of participants in the program go on to become academic faculty members in dentistry. As dental faculty grow older, there is a looming critical shortage of dental faculty. The dental student research program at Iowa has been an effective tool to support pathways from dental school to academic dentistry.

A look back at Research Day 2023



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“Sometimes faculty and staff don’t realize the kind of review that our team provides so that we can make our grants as competitive as possible,” explained Mikaela Rhoads, research support specialist on the grant administration team for Iowa Dentistry. “Our goal is to free up our faculty to focus on the science in their grants,” she added.

When faculty submit materials to the grant administration team well before the deadline, the team has the space and time to provide stronger reviews and more support. This kind of support is important for faculty at all stages of their career, but it has proven especially valuable to those just starting their research.

NIH funding is extremely competitive, and a small percentage of grants submitted end up being funded.

“We train new faculty and research fellows to help them navigate the complicated NIH system, so that they understand the process, the grants available and their requirements, and are able to develop competitive grant applications with an appropriate budget,” Rhoads explained.

The grant administration team's process helps to increase the success rate of Iowa Dentistry’s grant applications. The results have been promising. The team submits approximately \$55 million in grant proposals to various funding agencies annually. Since 2020, Iowa Dentistry’s research funding has doubled, nearing approximately \$10 million per year.

The grant administration team also ensures that all requirements are met after an award is granted.

Maria Bertorello, research support specialist on the grant administration team, is the primary support for post-award grant administration. This work involves requests for extensions, completing reports for grants in progress or closed, adding subawards for grants, and making financial projections.

Given the flurry of activity, the increase in number of grants submitted, and recent increases in the number of grants funded, the workload for the team has significantly increased over the last several years. That’s one reason the team expanded from one person prior to 2020 to the three-person team now. Yet the work continues to expand, and the team continues to support further research success.



Associate Dean for Research Jin Xie is discussing grant administration with Ann Lawler. Xie routinely champions the research administration and support team, and he has been instrumental in expanding the research administration team from one person in 2019 to its three people currently. As a result, the entire research enterprise has been able to expand, submitting more grants for external funding, being awarded more external funding, and managing a greater number of grants than ever before.

Their secret: “We work really hard,” said Lawler, “and we work really well together,” added Rhoads, “and we rely on one another,” said Bertorello.

Together, they form a strong, coherent team. As another cycle of grant submissions come and goes, please take the time to express your appreciation for the excellent work of the grant administration team. They are juggling multiple projects, at various stages of completion, from faculty and research fellows at all stages of their careers.

“The grant administration team is superb,” said Xian Jin Xie, associate dean for research in Iowa Dentistry, “and our research successes are due in no small part to their on-going efforts to support our faculty and researchers.”

Acknowledgments

We extend our grateful acknowledgment to the following members of our College of Dentistry family:

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| Clark Stanford | Scott Arneson | Barb Colbert |
| Susan Hackert | Brian Howe | Kecia Leary |
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| Sherry Timmons | Xian Jin Xie | |

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Judges and Moderators for the Iowa Section of the AADOCR Research Day



Dental research images for the cover were graciously contributed by Erika Araujo, Austin Green, Ariene Leme Kraus, Cristina Vidal, and Jinmei Zhang.

We extend our grateful acknowledgment to the following sponsors and supporters:



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