

# IOWA

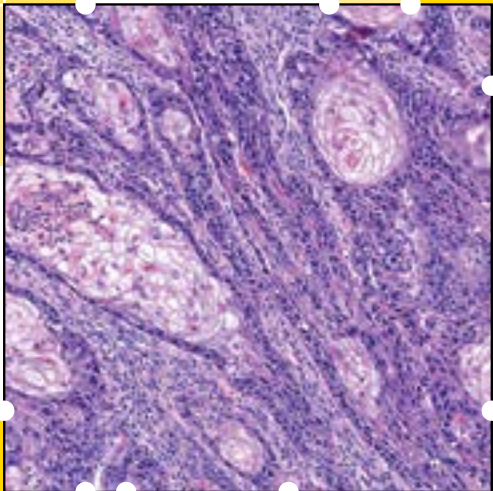
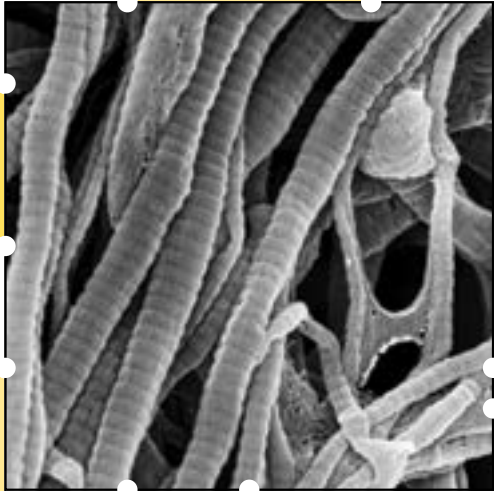
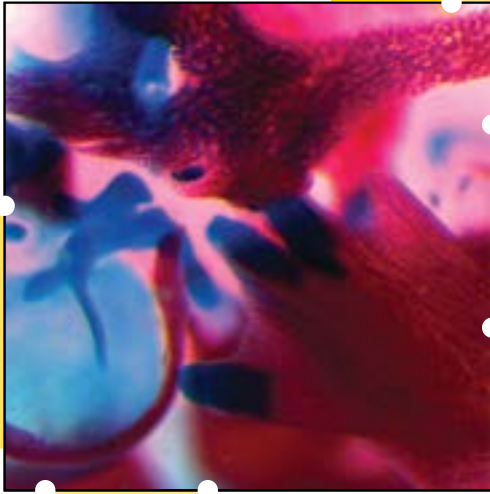
*College of Dentistry and Dental Clinics*

## IOWA SECTION OF THE AMERICAN ASSOCIATION FOR DENTAL, ORAL, AND CRANIOFACIAL RESEARCH

# 70<sup>th</sup> ANNUAL MEETING

Leveraging Biosystems  
and Revolutionizing  
Oral and Craniofacial Research

# 2023





Dr. Yvonne Hernandez-Kapila  
DDS, PhD

## Our Keynote Speaker –

Yvonne Hernandez-Kapila is the associate dean for research at UCLA, where she is the Felix and Mildred Yip Endowed Professor. She has produced seminal discoveries in the cell and molecular biological mechanisms governing periodontal disease pathogenesis and oral cancer carcinogenesis, and has conducting important research related to periodontal disease, oral cancer, caries assessment, fluorosis assessment, and oral health and pain research. As a clinician scientist, her research team has been continuously funded by the NIH for 25 years and has authored over 140 peer-reviewed publications in leading journals in her fields. She is also a leader in academic dentistry, both at UCLA, for the larger University of California system, and the NIH, NCI, and American Academy of Periodontology.

### Keynote Address:

#### *Harnessing the Power of the Oral Microbiome to Address Oral Diseases*

Harnessing knowledge about the oral microbiome and probiotics are useful tools for addressing oral diseases. The presentation will examine research on the interactions host and the oral microbiome for oral diseases, while examining interventions to improve patient outcomes for those who suffer or are likely to suffer from oral diseases.

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## Our Featured Speakers –



Juhi Uttamani,  
BDS, PhD

Dr. Juhi Uttamani joined the UI College of Dentistry Department of Periodontics in 2022 after completing her Bachelors of Dental Surgery from Mumbai, India and PhD in Oral sciences with an advanced postgraduate certificate in periodontology from University Of Illinois at Chicago. She was also a postdoctoral research fellow at UIC, Chicago.

### Featured Address:

#### *miRNAs in Periodontal Health and Disease*

The etiology of periodontal disease is polymicrobial, polygenetic, and modulated by other environmental factors and systemic conditions. MicroRNAs (miRNAs) participate in the epigenetically mediated RNAi mechanism to regulate post-transcriptional gene expression and multiple physiological and pathophysiologic functions. It is likely that miRNAs regulate inflammatory responses, and studies including ours have identified miRNA changes that fine-tune the regulation of biological processes essential in maintaining periodontal tissue integrity. Thus, identifying and characterizing miRNA signatures for periodontal health and disease is vital.

Dr. Eric Van Otterloo obtained his Ph.D. in Anatomy and Cell Biology, from the University of Iowa in 2012. Subsequently, he was a Postdoctoral Fellow and Research Associate from 2012-2019 in the Department of Craniofacial Biology at the University of Colorado, School of Dental Medicine, before transitioning back to the UI. Dr. Van Otterloo joined the UI College of Dentistry, and the Iowa Institute for Oral Health Research, in 2019 as an assistant professor in the Department of Periodontics.

### Featured Address:

#### *Deciphering Tissue Level Programs of Craniofacial and Dental Development*

Our genes orchestrate the precise organization of our face and teeth during embryonic development. While genetic differences underly craniofacial variation, they can also lead to birth defects. One example of such a gene is TFAP2, although how it controls these processes is unclear. Through mouse genetics and cutting-edge molecular techniques, our lab is uncovering how this gene guides craniofacial and dental development. Our hope is to leverage this information for future therapeutic approaches.



Eric VanOtterloo,  
PhD

## Iowa's Groundbreaking Oral Cancer Program

When Xian Jin Xie came to Iowa as the director of the Division of Biostatistics and Computational Biology in 2017, he had the goal of making Iowa a global leader in oral cavity cancer research.

"Our excellent faculty across campus—not just in dentistry, but also in medicine, pharmacy, and public health—and having access to the infrastructure and expertise of the Holden Comprehensive Cancer Center made Iowa the perfect place to build the oral cavity cancer program. The spirit of teamwork among our researchers is second to none," said Xie, who is now the associate dean for research at the University of Iowa College of Dentistry.

But building this new oral cavity cancer program (OCP) would require establishing a common vision and mission, developing new structures to harness and bring together experts from across campus, and creating a plan to execute what was then an inchoate research program in oral cavity cancer.

Fast forward five years later, and the program is off and running.

*Aliasger Salem is the lead for one of the Oral Cancer Program's research projects on preventing pre-malignant tumors from becoming malignant.*

### Common Vision and Mission

Although there are numerous specialized research centers that focus on head and neck cancer generally, to date there are none that specialize in oral cavity cancer research specifically. Tumors in the oral cavity, which affect almost 400,000 worldwide people per year, are most often found on the tongue, at the bottom of the mouth or lower lip, but sometimes in other places too. Oral squamous cell carcinoma (OSCC) represents about 90% of oral cavity tumors. Furthermore, Iowa's age-adjusted oral cavity cancer rate is the highest in the United States (2015-2019).

The risk factors for such tumors differ from head and neck cancer more generally. The most common forms of tumors in these areas are, for instance, strongly correlated with alcohol and tobacco use, but throat cancer is strongly correlated with human papillomavirus (HPV) instead.

Clinically, the conditions are also distinct. Oral cavity tumors are often first diagnosed in dental clinics, and in fact, many pre-malignant tumors are found, since oral cavity cancer screening is routine in dental clinics. This makes treatments which can prevent tumors from becoming malignant especially important in oral cavity cancer research.



*Emily Lanzel is an oral pathologist and dentist who is helping establish and further develop collaborative partnerships between the College of Dentistry and other units on campus in oral cancer research.*

Thus, one research team is exploring use of probiotics to reduce the risk of tumors developing in the oral cavity.

Another research team is also working on a vaccine that could potentially prevent pre-malignant tumors from becoming malignant.

"There is an urgent need to identify effective local and/or systemic therapies as an adjuvant to surgery to lower the risk of recurrence and malignant transformation," explained Aliasger Salem, professor of pharmacy, associate vice president of research administration at the University of Iowa, and the lead for one of three main research teams in the OCP.

"Our preliminary data has shown promising anti-tumor immune responses using in situ immunization with a toll-like receptor 9 (TLR9) agonist, and we hypothesize that in situ immunization with this agonist will trigger cytotoxic T cell infiltration thereby reducing oral leukoplakia lesion recurrence and/or malignant transformation to oral squamous cell carcinoma," he added.

Of course, OSCC does occur and needs to be treated. A third team is exploring methods to improve treatment and bone regeneration after surgery using bioengineering and miRNAs.

Thus, Iowa's focus on oral cavity cancer specifically fills an important gap in the current research landscape and will improve healthcare in Iowa.



*As the director of the Holden Comprehensive Cancer Center, George Weiner brings a wealth of experience and knowledge in support of the Oral Cancer Program.*

**Since Fall of 2018, the Oral Cancer Center has held 40+ all-OCP meetings with 30+ faculty researchers participating!**



Researchers in the Oral Cancer Program come from across campus. Andean Simons-Burnett is an associate professor of pathology, specializing in oral cancer research in the Carver College of Medicine and Holden Comprehensive Cancer Center.

Holden Comprehensive Cancer Center (HCCC) has those resources, including the ability to conduct Phase 1 clinical trials and to process samples within their infrastructure. But it was important to set up structures in place to support on-going collaborations with the HCCC to effectively draw from experts across campus who were doing effective and relevant research in the area.

“It is a pleasure working with the College of Dentistry to establish Iowa as a leader in oral cavity cancer research. The Holden Comprehensive Cancer Center includes investigators from 6 colleges and 36 departments, and dentistry colleagues are important contributors,” said George Weiner, professor of internal medicine and director of the Holden Comprehensive Cancer Center.

### Executing the Plan

With the vision, mission, and infrastructure in place, Iowa was ready to get started. The initial meetings began in the fall of 2018 and continue in earnest to this day.

In addition to numerous and regularly weekly and biweekly meetings for subgroups, the full OCP team met every other week to update the entire group on overall and subgroup progress.

Through this work, the team refined its goals, research teams and projects, and began developing the infrastructure to support an on-going oral cavity cancer program at Iowa.

“Oral Cancer Program” continued on page 52 ▶

### Developing the Infrastructure

Once the mission and vision were established for the program, it was vital to develop the infrastructure to support the unique features of an effective oral cavity cancer program. Drawing together researchers from across Iowa’s campus was vital.

“Oral cancer diagnosis and treatment are a primary concern for dentistry as a whole and of course particularly for the University of Iowa and its research mission,” explained Emily Lanzel, clinical assistant professor of oral pathology, “and a true multidisciplinary collaboration is vital to produce the most effective research.”

One part of this kind of collaboration in oral cavity cancer research requires the incorporation of dentists and dental researchers into the research team. Dentists are the primary source of patient referrals for oral cavity cancer since they universally screen patients for signs and symptoms of oral cavity cancer.

“The University of Iowa College of Dentistry providers strive to detect and treat, if necessary, premalignant as well as malignant diseases of the oral cavity, and it’s cohort of patients provide a large enough patient pool to derive past, present, and prospective data as needed,” Lanzel added.

However, connecting these dental providers to the robust resources of larger cancer research networks is also essential to support the long-term viability and sustainability of such a research program. The



Fabricio Teixeira is professor and head of the Department of Endodontics and a collegiate leader in securing industry-sponsored grants, which produce benefits for the dental profession, the college, and our industry partners.

### Industry-University Partnership to Evaluate Healing of Apical Periodontitis

Building on the University of Iowa College of Dentistry’s longstanding experience of university-industry collaboration, Fabricio Teixeira, professor and head of the Department of Endodontics, is working with the medical equipment manufacturer SONENDO of California, to evaluate a new piece of specialized equipment that SONENDO has developed called GentleWave.

“It is paramount that researchers maintain their autonomy from external sponsors,” Teixeira said, “since it protects both the integrity of the researcher and the reputation of the company.”

The traditional treatment for infections that cause apical periodontitis is generally a standard root canal treatment using needles and syringes to flush the microbes and debris in the root canal using suction and water irrigation. In most cases, apical periodontitis will heal if the root canal system is adequately disinfected.

SONENDO’s GentleWave system uses soundwaves rather than fluids to disinfect the root canal system. The process can be completed in eight minutes, and the company has indicated that it has evidence that their process results in faster healing times for patients.

Teixeira and his team are providing an external and independent evaluation of the working time and post-operative pain levels patients undergo after the GentleWave procedure. Notably, the team will estimate the healing of apical periodontitis using CBCT technology and whether the GentleWave treatment outcomes are superior to current disinfection approaches.

In addition to federal and private-foundation grants, industry-sponsored grants provide an essential revenue stream to support and encourage faculty research at the college.

“Faculty have to be open-minded about the opportunities and the challenges of industry grants,” Teixeira explained.

“Teixeira” continued on page 52 ▶



*Aline Petrin is an assistant professor of orthodontics, and a rising star in the college and in genetic research on orofacial clefts.*

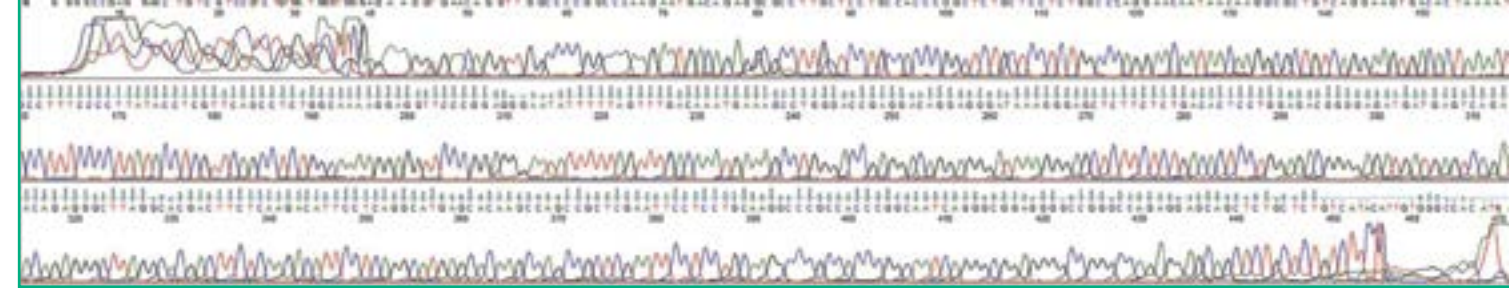
## Twins and the Causes of Orofacial Clefting

When parents dress identical twins alike, it can be nearly impossible to tell them apart. Aline Petrin, assistant professor of orthodontics at the University of Iowa, is harnessing the genetic and epigenetic sameness of identical twins to shed new light on the causes of orofacial clefts.

Orofacial clefts are conditions that affects 1.25 out of every 1,000 live births, and it can significantly impact the health and wellbeing of those affected. For monozygotic (identical) twins, there are a few instances where one twin will have orofacial clefting and the other twin will not. These clear and physically observable differences are known as differences in phenotype. For these discordant identical twins, genetics alone cannot be the cause of clefting since they are genetically identical.

Instead, Petrin and her team are exploring if epigenetic factors which influence gene expression

*Her research on the epigenetics of orofacial clefts highlights the importance of DNA methylation as a causal factor responsible for orofacial clefts among twins.*



*The graph shows DNA sequencing from human oral epithelial cell lines that Petrin and her team use to validate the results from their twins studies.*

without altering the genetic code could be the root cause of discordancy between the cleft phenotypes in these twins.

Drawing from the 30 pairs of discordant identical twins, Petrin has identified DNA methylation—a biochemical reaction in which a small molecule is added to DNA—as a primary source that accounts for clefting, although the precise mechanism is still unknown.

It may be that very early environmental factors explain why one twin has clefting and the other does not. But if it is environmental, it would most likely have occurred very early on during a pregnancy during the twinning process, since the methylation profiles of twins are very similar, and most aspects of their environment are the same.

The team has also identified 40 dizygotic (fraternal) twins where one twin has orofacial clefting and the other twin does not. These twins augment the earlier research and has allowed the team to tune their examination of the epigenetic processes more carefully.

Based on their findings, Petrin and her colleagues are identifying what happens with small changes are made using CRISPR-based gene editing to manipulate DNA methylation and subsequent gene expression. This allows them to see the results of small epigenetic changes in real time and thus learn more about the types of cells involved and how they are functioning.

The future is bright for Petrin and her research, who recently accepted a position as assistant professor of orthodontics at the University of Iowa.

“Dr. Petrin is a rising star at the College of Dentistry, bringing her broad background in all aspects of human genetics research,” gushed Lina Moreno, chair and associate professor of orthodontics at Iowa and one of Petrin’s mentors.

Moreno points to Petrin’s own mentoring as one way that she is bringing her research experience to benefit Iowa students.

“In just a short amount of time, Dr. Petrin has already mentored undergraduate students, predocs and postdocs very successfully all of whom have presented their work at the national meetings with distinction,” Moreno said.

Petrin’s success at Iowa began when she met Jeff Murray, professor of pediatrics at the University of Iowa, during a research trip to Brazil, where Petrin completed her PhD. Based on that meeting, he invited Petrin to come conduct research in his laboratory in Iowa. Later, Lina Moreno, chair and associate professor of orthodontics at Iowa, began mentoring Petrin as a postdoctoral researcher.

Through that process, Petrin became involved with the training program in the Iowa Institute for Oral Health Research for doctoral students and postdoctoral researchers, which helped prepare her for the grant writing process. This process ultimately resulted in her receiving a post-doctoral training grant from the National Institute for Dental and Craniofacial Research.

Petrin also participated in the American Association for Dental, Oral, and Craniofacial Research’s MIND the Future program, which is designed to encourage and support career development from persons from underrepresented backgrounds.

Petrin is quick to express her appreciation for these opportunities and her mentors, like Moreno.

“Dr. Moreno has been wonderful and is a great clinician and scientist—she knows genetics and clefting so well, and her clinical expertise has shown me the complementary way of thinking between bench and the chair,” Petrin said.

Whether its conducting collaborative and innovative research or mentoring the next generation of oral scientists and clinicians, Petrin is making an impact.

## Dental Student Researcher Studies Craniofacial Biology at Iowa

Some people take a while to figure out what they want to do in life. Other people know early on. Third-year dental student Mary Young is in the latter camp.

“I was always interested in dentistry and knew that I wanted to be in a health care-related field and that I enjoyed working with my hands,” Young explained.

Although she didn’t have any specific connections to the dental profession except for her “super-involved” dentist, Shawn Hedlund of Melrose Dental Office in Iowa City, she made the most of what was available. Hedlund provided a great deal of insight into the process of getting where Young wanted to go, including encouraging her to get critical experiences in dental clinics, shadowing dentists, and so forth.

As part of this process, she also began volunteering in scientific research laboratories in 8th grade with Gina Schatteman and later with Martine Dunnwald, and through those early connections, she began participating in Azeez Butali’s laboratory in high school, and continued as an undergraduate at Iowa

and as a dental student throughout dental school, where Teresa Marshall also helped mentor her as part of the Dental Student Research Program.

Cleft lip and palate accounts for a significant proportion of neonatal birth defects in Africa, but there is very little data on the genetic causes of the condition for this population. Since 2010, the Butali laboratory has established itself as the worldwide leader in research on the genetic characteristics related to orofacial cleft lip and palate in African populations. This population is the least studied population in the world, and yet it critically impacts many members of the African community. The Butali research team is not content to only make exciting discoveries, but they are also committed to bringing their findings to bear on pressing social, cultural, and religious challenges that sometimes serve as barriers to making positive differences in the lives of those affected by their research.

Young began working in the Butali lab in 2015 in the summer before her senior year of high school. Each year since, she continued to build skills in DNA extraction, genome sequencing, conducting experiments in animal models, targeted gene sequencing, and bioinformatics research.



*Young enjoys bench work with DNA as a member of the Butali laboratory..*

*Mary Young is a third-year dental student at the University of Iowa, and she has worked with Azeez Butali, professor of oral pathology, radiology, and medicine throughout her years at Iowa while in high school, as an undergraduate, and in dental school.*



Based on Young’s experience, Butali and Young have established something of a pattern for how young researchers develop in his lab with a clear progression in skill development and research competence.

“Back then, Dr. Butali was establishing training protocols for young members of his lab, and it all began with me sitting at the bench, jamming out to music, and extracting DNA from hundreds of samples. It was perfect!” said Young with a grin.

This pattern has served Young well. As a member of the Butali research team, she has received numerous prestigious awards for her work, including a 2021 Student Research Fellowship from the American Association for Dental, Oral, and Craniofacial Research, a 2022 National Institutes of Health Summer Dental Research Award, and most recently, she was selected to be featured in the 2023 Office of the Vice President for Research’s Dare to Discover Banner campaign.

While at the National Institutes of Health in Maryland this past summer, Young’s project continued to focus on craniofacial biology, albeit in a different area

than clefting in African populations. This project examines the mechanisms behind neural crest cell fate decisions and helps identify candidate targets for improved bone regeneration (see Presentation #8 in this booklet).

Butali has long commended Young’s dedication, hard work, and commitment.

“I am so proud of all that Mary has already accomplished, and I know that she will do great things and be an excellent representative for our college,” Butali said with pride as he added, “and I am fully committed to doing my part to ensure that Mary is able to become an independent, fully-funded dental scientist.”

Young isn’t sure what the future holds for her now.

“Whatever I end up doing, I know that I love seeing patients, doing dentistry, and contributing to evidence-based care. With all that I will learn clinically and my continued work in orofacial clefting genetics, I hope to combine both worlds into clinical dentistry that improves the lives of others,” she said.

Dear members of the College community:

Thank you for your participation in the 70th Anniversary of the University of Iowa College of Dentistry's Iowa Section of the AADOCR, February 14, 2023.

Discovery science is crucial to better health and longevity of dentistry as a scientific profession. Iowa is a leader in dental, oral, and craniofacial research, producing groundbreaking discoveries fundamentally changing dentistry and related subfields. We continue our on-going legacy today. Our research success over the last few years has been remarkable, and we are poised for even greater success. This is happening via a strong mix of established researchers and junior faculty, basic, translational and clinical research. This speaks to the long-term health and vitality of our research and discovery enterprise.

The research presented today provides a glimpse of these efforts and demonstrates the rich diversity of research discovery and impact on the college, the University and our profession. The research presented is wonderful, be it a student presenting for the first time, a graduate student, post-doc or an established scholar. I want to thank our presenters and the planning committee for their extensive contributions to these efforts.

We are honored to host Dr. Yvonne Hernandez-Kapila, associate dean for research at UCLA, as our keynote speaker. Dr. Hernandez-Kapila's career demonstrates the power of transdisciplinary collaboration where basic discovery science impacts on understanding the rich interactions between the host and a complex microbial and viral biofilm. Her work has brought together very diverse teams of scholars and her mentoring has in turn, established a strong set of investigators continuing her legacy in the field of Periodontology.

Hernandez-Kapila's research and discovery in the field of oral cancer carcinogenesis closely aligns to the oral cancer related research projects currently underway at the college. This day is an opportunity for us to showcase our research and discovery demonstrating impacts on education, service, research, and patient care. 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. Over the last two years, the college has achieved unprecedented success in research with a record number of funds awarded and a record number of grant applications.


This upward trajectory is also evident in the College developing, in collaboration with numerous other colleges across campus, the first-of-its-kind Oral Cavity Cancer Program. The seed grant program will begin its third year in operation, and many of the initially funded projects are beginning to bear fruit as the researchers secure external grant funding for those projects. The college's Clinical/Dental Education Research Initiative Support Program (CRISP) is continuing for a second year, which demonstrates the college's commitment to improving clinical and dental education research.

Our excellent faculty, NIH T90/R90 training program, Biostatistics and Computational Biology unit, Clinical Research Office and graduate and pre-doctoral Student Research Programs also continue to thrive in tandem with these recent successes.

This year we are honored to have Dr. Yvonne Hernandez-Kapila, the Felix and Mildred Yip Endowed Chair and associate dean for research at UCLA, as our keynote speaker. Dr. Hernandez-Kapila is a global leader in cell and molecular biology, investigating mechanisms that govern periodontal disease pathogenesis and oral cancer carcinogenesis. Our featured speakers for 2023 are Drs. Eric Van Otterloo and Juhi Uttamani, both assistant professors in the College of Dentistry.

As we look to the future, we are poised for even greater research success for our college, but you are the ones who make it happen. This day is an opportunity for us to show the best of our basic, clinical and evidenced-based research studies. Our future is bright thanks to all of you!

Warmest Regards,



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 are very pleased to welcome you to our 70th Annual Meeting “Leveraging Biosystems and Revolutionizing Oral and Craniofacial Research.” As we fully return to a post-pandemic in-person meeting to present novel research discoveries and create and strengthen new opportunities to collaborate with colleagues.

We are honored to have Dr. Yvonne Hernandez-Kapila, an expert in periodontal disease and oral cancer, as our keynote speaker, alongside Iowa’s own Dr. Eric Van Otterloo and Dr. Juhi Uttamani as featured speakers. Each of these researchers are conducting exciting work on biosystems with the aim of changing how we do oral and craniofacial research more broadly, and they encompass a wide spectrum of basic scientific, translational, and clinical research.

Many of our oral and poster presentations are further developing these areas of study, including numerous graduate student and dental student researchers. This body scholarship represents some of the most exciting areas of inquiry in the dental academy and oral health research, and the University of Iowa’s breadth and depth of research is well represented in this annual meeting.

As part of the leadership team for the 2023 Meeting of the Iowa Section of the AADOCR, we welcome you to our annual meeting. We hope you find it as rewarding and promising as we do!

Sincerely,



Emily Lanzel, DDS, MS  
President, Iowa Section of the AADOCR  
Clinical Assistant Professor  
Department of Oral Pathology, Radiology, & Medicine



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



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



Hongli Sun, PhD  
Secretary/Treasurer, Iowa Section of the AADOCR  
Associate Professor  
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 AADOCR. We appreciate our joint efforts to advance the exchange of ideas, research and education, which strengthens oral health global initiatives here and abroad, as we persevere through the pandemic and achieve excellence in dental, oral, and craniofacial research.

### 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 have presented their research in-person or virtually at the Annual Meetings of the Iowa Section of the AADOCR since 2018. This partnership helped strengthen our commitment to the exchange of ideas, research, teaching, and training opportunities for all of our faculty and students.
- 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 had several joint research projects and collaborations including:
  1. A published collaborative research project with Dr. Yi Su from Chongqing Medical University. Spent culture supernatant of *Streptococcus gordonii* mitigates inflammation of human periodontal cells and inhibits proliferation of pathogenic oral microbes. Shu Y, Upara C, Ding Q, Zhu M, Zeng E, Banas JA, Hong L. *J Periodontol.* 2022 Nov 12. doi: 10.1002/JPER.22-0333.
  2. Continued collaboration on a clinical research project with Dr. Yi Shu with likely future in-person collaborations.
- We are pleased to welcome our Chongqing collaborators to the Annual Meeting of the Iowa Section of the AADOCR held on February 14, 2023. We are advancing our exchange of ideas, research and education, which strengthens oral health global initiatives here and abroad.



## Program

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

70th Annual Meeting, Tuesday, February 14th, 2023

- 7:30 a.m.**                    **Breakfast**  
(First Floor Link)
- Morning addresses will be presented in the Galagan Auditoria
- 8:00 a.m.**                    **Welcome Address**  
Dr. Clark Stanford
- 8:10 a.m.**                    **Welcome Address, and Introduction of Featured Speakers**  
Dr. Jin Xie
- 8:20 a.m.**                    **Featured Speaker**  
Dr. Juhi Uttamani  
*"miRNAs in Periodontal Health and Disease"*
- 8:37 a.m.**                    **Featured Speaker**  
Dr. Eric VanOtterloo  
*"Deciphering Tissue Level Programs of Craniofacial and Dental Development"*
- 8:55 a.m.**                    **Keynote Speaker Introduction**  
Dr. Sukirth Ganesan
- 9:00 a.m.**                    **Keynote Address**  
Dr. Yvonne Hernandez-Kapila  
*"Harnessing the Power of the Oral Microbiome to Address Oral Diseases"*
- 9:45 a.m.**                    **Break**
- 10:00 a.m. - 12:00 p.m.**    **Oral Presentations**  
(Galagan A, Galagan B, Galagan C, W205, & N212 Oral-B classroom)
- 11:45 a.m. - 12:45 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. Maxillary Shape Variations in Unaffected Relatives of Individuals with Clefts



**Luke T. Hovey**<sup>1</sup>, L. Sarment<sup>1</sup>, M. Hemann<sup>189</sup>, S. Miller<sup>44</sup>, T. Soejima<sup>59</sup>, T. Wiedow<sup>1</sup>, E. Bell<sup>189</sup>, C. Restrepo<sup>146</sup>, C. Valencia Ramirez<sup>146</sup>, A. Butali<sup>1</sup>, C. Buxo<sup>60</sup>, R. Long<sup>178</sup>, B. Howe<sup>1</sup>, M.L. Marazita<sup>59</sup>, S. Weinberg<sup>59</sup>, *L.M. Moreno Uribe*<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA; <sup>44</sup>University of Illinois, CHICAGO, Chicago, IL; <sup>59</sup>University of Pittsburgh, Pittsburg, PA; <sup>60</sup>University of Puerto Rico, San Juan, PR; <sup>146</sup>Clinica Noel, Medellin, Colombia; <sup>178</sup>Lancaster Cleft Palate Clinic, Lancaster, PA, US; <sup>189</sup>Private Practice, Minnesota

**Objective:** Individuals with orofacial clefts (OFCs) present dento-alveolar shape (DAS) anomalies associated with OFC genetic risk and due to the overt cleft and surgical scarring. Distinguishing between these different etiologies is key to improving cleft care. Studies of seemingly unaffected family members (UFM) of affected individuals with whom they share OFC genetic risk have unraveled aspects of the OFC phenotypic spectrum that are not related to the overt cleft but to plausible OFC genetic risk. We evaluated maxillary DAS differences between UFM and controls with no cleft history.

**Methods:** First molar-first molar maxillary arch scans (N=1,308) were landmarked with eighty landmarks, located on the gingival margins and occlusal surfaces of the arches. 3D coordinates were adjusted using a Procrustes fit and submitted to a canonical variate analysis to compare DAS differences between UFM and controls. DAS was also compared between UFM of cases with different cleft types: cleft lip (CL), cleft lip and palate (CLP) and cleft palate (CP). Statistical significance was assessed via permutation tests with 1,000 iterations.

**Results:** Maxillary DAS was significantly different (P<.05) between UFM and controls. Major shape differences between UFM and controls include a tendency for UFM to have facially angled incisors, buccally tipped molars and constrained arches in the A-P dimension. Comparisons between UFM of different cleft types were also significantly different (P<.05). UFM of cleft palate only individuals tended to have more facially angled incisors, shorter posterior teeth, and more lingually tipped molars when compared to UFM of CLP and CL individuals. Although significant, UFM of CLP and CL individuals had less distinctive shape variation.

**Conclusion:** DAS differs between UFM and controls indicating abnormal shape associated with OFC genetic risk. Comparisons between cleft types showed more distinct DAS differences when comparing UFM of CP individuals and UFM of CLP and CL individuals.

Supported by: The University of Iowa Dental Student Research Program.

#### 2. Developmental Enamel Defects in Non-Syndromic Orofacial Clefting



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**Objective:** Children with Non-syndromic Orofacial clefts (OFC) often show an increased rate of developmental enamel defects (DED), such as hypoplasia and hypocalcification. Few prior studies discussed the location of the defects within the clinical crown and if the location can be associated with the development of a cleft defect or corrective surgery sequelae. This study aims to characterize the developmental location of hypoplasia and hypocalcification in children with OFC compared to controls. Findings will assist in the understanding of the underlying etiology of DEDs and will also help to provide early interventions, reduce adverse effects, and improve treatment outcomes.

**Methods:** The study included 255 subjects with OFC and 1,273 control subjects recruited internationally, as part of an NIH multicenter OFC study. Hypocalcification and/or hypoplasia were identified from intraoral photographs using an electronic intake form. The defect location was classified as incisal, middle, or gingival third of the clinical crowns in primary and permanent dentitions. Case-Control differences were assessed using Fisher's Exact test and Bonferroni correction was set at p-value=8.68E-5.

Continued ►

**Results:** Subjects with OFC showed significantly more hypoplasia vs. controls on FDI teeth #21 Gingival,  $p=1.6E-5$ , and #11 and #21 Middle,  $p=2.9E-6$  and  $p=8.0E-11$ ; respectively. Subjects with OFC showed significantly more diffuse hypocalcification vs. controls in the middle third (#11,21) and Incisal third (#11,21,31,32,33,41,42,43,44) with p-values ranging from  $1.1E-6$  to  $5.0E-11$ . Controls had significantly more diffuse hypocalcification in the gingival third (#14,16,22,24,26) with p-values ranging from  $1.1E-6$  to  $2.0E-13$ . No significant differences were found in the primary dentition or demarcated hypocalcification. Additional evaluation by sex or cleft-type is underway.

**Conclusion:** These findings indicate that subjects with OFC have more hypoplasia in the gingival and middle-thirds and diffuse hypocalcification in the incisal and middle-thirds of permanent teeth. This could result from a shared underlying etiology with the cleft defect or downstream effects of the corrective surgery on the surrounding dentition.

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### 3. Novel Risk Loci and Processes for Non-Syndromic Orofacial Clefts



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**Objective:** Non-syndromic orofacial clefts (NSOFCs) are the most common craniofacial birth defects affecting 1 in 1000 live births worldwide. The etiology is multifactorial, and all the risk loci identified from published genome-wide association studies (GWASs) on NSOFCs explain only a small proportion (20-30%) of the estimated heritability. GWAS signals with suggestive associations in smaller cohorts tend to achieve genome-wide significance in larger cohorts. Further, the cumulative effects of multiple SNPs in functionally related genes have also been implicated. Thus, we replicated GWAS signals with suggestive associations and performed a pathway-based analysis to discover significant loci and biological processes for NSOFCs.

**Methods:** We genotyped 120 SNPs with suggestive associations ( $E-05 = P = E-08$ ) from the African NSOFCs GWAS in an independent cohort of 379 cases and 449 controls from Nigeria, Ghana, and Ethiopia. Performed allelic association analyses adjusting for the top principal components (PCs) and meta-analyzed with the GWAS results. Further, we performed a pathway-based analysis using our GWAS summary statistics data adjusting for linkage disequilibrium (LD) between SNPs, gene size, and pathway size.

**Results:** We found significant associations for rs9542663 (chr13q21) and rs17242358 (chr8q24) in the cleft lip with or without cleft palate (CL/P) cohort (PCL/P =  $6.4E-05$  and PCL/P =  $5E-04$  respectively) While rs28713678 (chr9q22) showed significant association with cleft palate only (CPO) (PCPO =  $5.8E-05$ ). On meta-analysis, the SNPs rs28713678 and rs9542663 showed significant associations with CPO ( $P = 3.34E-09$  and  $1.36E-08$ ; respectively), while rs17242358 was significantly associated with CL/P (PCL/P =  $7.9E-09$ ). The rs28713678 and rs9542663 lie near the PTCH1 and DACH1 genes, respectively. The pathway-based analysis identified cell adhesion and neurogenesis as the most significantly enriched processes.

**Conclusion:** The 8q24 is a widely reported cleft candidate region, while PTCH1 and DACH1 are cleft candidate genes. Additionally, we found evidence for cell adhesion and neurogenesis in lip and palate formation.

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### 4. Deciphering the Tissue Specific Role of MEMO1 in Craniofacial Development



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**Objective:** Embryonic development of craniofacial structures relies on the interaction of various cell and tissue types. In particular, the interaction of neural crest-derived mesenchyme with the mesoderm-derived endothelium (i.e., vasculature) plays an obligatory role in proper bone development and mineralization. Despite neural crest and vascular defects underlying craniofacial anomalies, our understanding of how these tissues interact during development remains limited.

We previously identified a novel role for Mediator of ERBB2-driven Cell Motility 1 (Memo1) in craniofacial development. Global loss of Memo1 resulted in various defects, including hypo-mineralization of the craniofacial skeleton, fully penetrant cleft secondary palate, and widespread vascular abnormalities. Interestingly, while neural crest cell-specific deletion of Memo1 recapitulated some aspects of global loss of Memo1, several defects were less apparent, suggesting MEMO1 functions in tissues outside

the neural crest during craniofacial development. Further, molecular profiling of craniofacial elements in global Memo1 mutants identified significantly altered Vegfa expression, coupled with disrupted vascular development, relative to controls. Based on these observations, we hypothesized that MEMO1 functions within the endothelial-derived vasculature, and this function is necessary for proper craniofacial development.

**Methods:** To test our hypothesis, we generated a Memo1 endothelial-specific knockout mouse model. We are currently utilizing  $\mu$ CT, cell sorting coupled with gene expression profiling to quantify the impact endothelial loss of Memo1 has on craniofacial bone development and mineralization, MEMO1's molecular role in craniofacial endothelial cells, and MEMO1's functional role in endothelial cells, respectively.

**Results:** Like global Memo1 knockouts, Memo1 endothelial knockouts develop vascular hemorrhaging and edema with variable penetrance. Vascular defects are accompanied by gross alterations to craniofacial shape and mineralization defects.

**Conclusion:** Our findings expand the repertoire of MEMO1's function during craniofacial development by illuminating its role in an additional cell type — endothelial cells. Further work aims to identify how MEMO1's endothelial function influences cranial neural crest cell development and how these interactions culminate in the formation of facial structures.

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### 5. Community Gatekeepers and Genomic Risk Communication in Cleft Research



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**Objective:** In Africa, community gatekeepers exert considerable influence on the decision-making process and outcomes of their members. However, little is known about their opinions on genomics testing and its outcomes. We also do not know how these opinions impact findings from genomic testing, particularly in the context of orofacial clefts (OFCs). Thus, this study aims

to explore the perspective of community gatekeepers in collaborative decision-making for genomics risk communication (GRC) in a minority cleft cohort.

**Methods:** Twenty-five focus group discussions (n=214) were conducted among religious, ethnic, community leaders and traditional birth attendants in Lagos, Nigeria to collect information on participants' opinions on GRC. Using thematic analysis, codes generated from recorded transcripts were analyzed using N-Vivo.

**Results:** Three main themes were identified; support, willingness to collaborate and preferred learning style. Many participants believed their role is to support members as they receive results. They also believed that they were well positioned to help members better understand testing outcomes based on the level of trust their congregants have in them. However, they would need to be trained by medical experts to do this. Many preferred seminars and social gatherings to improve their genomics knowledge. Finally, mixed opinions were observed regarding which results to return. While some participants believed that only main findings should be returned, others supported the inclusion of secondary actionable findings.

**Conclusion:** Gatekeeper's willingness to support and help members with the understanding of genomic risk information is an important highlight of this work. Employing culturally sensitive public engagement methods is essential as we develop appropriate strategies to manage conflicting ideologies surrounding genomic information for individuals and different communities. Our findings are limited to groups included in the FGD and thus limits the generalizability of these findings across Africa. Further research encapsulating the socio-cultural uniqueness of the African population is thus needed for better representation.

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### 6. Whole Exome Sequencing Identifies Damaging Variants in Indonesians with Clefts



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**Objective:** Approximately 70% of cleft lip with or without palate and 50% of cleft palate cases are non-syndromic (nsOFC). The interaction between genomics, genetic and environmental factors have been implicated in the etiology of nsOFC. In the current study, we investigated the contributions of rare and novel genetic variants in known cleft genes using whole exome sequencing data of Indonesians with nsOFC.

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**Methods:** Whole exome sequencing was conducted on 6 individuals (5 with nsOFC and 1 unaffected). Called variants were annotated using Annovar. SNVs and indels in known cleft candidate genes were identified among the exonic variants. These variants were further filtered for novelty and rarity using MAF of 0 and 1% as reported in genome aggregate database, exome aggregate consortium and 1000 genome project database.

Deleteriousness of these filtered variants were predicted using polyphen, SIFT and a CADD score cutoff of  $\geq 15$ . Variants were prioritized if they were predicted to be deleterious by CADD, SIFT and PolyPhen2. ACMG criteria was also used to assess pathogenicity using VARSOME. Pathogenic amino acid changes effect on protein structure and function were identified using HOPE.

**Results:** We identified a very rare variant, rs1046263917 (MAF= 0.000016) in *CORIN* that was deleterious across all the 3 prediction tools and predicted to be likely damaging by HOPE. Other rare deleterious variants (MAF <1%) were also identified in 5 genes (*PAX7*, *SOS1*, *TULP4*, *MTHFD1L* and *MMACHC*).

**Conclusion:** Our study provides additional evidence for the role of *PAX7*, *TULP4*, *SOS1*, *MTHFD1L* and *MMACHC* genes in nsOFC development. We also identified a very rare variant in *CORIN*. This is the first time *CORIN* is implicated in nsOFC development in humans. The identification of rare variants (<1%) in these genes could explain the missing heritability for nsOFC not explained by common variants in GWAS.

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## 7. Epigenomic Analysis of Cleft Lip Laterality in Mirror Image Twins



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**Objective:** Nonsyndromic cleft lip (nsCL) is one of the most common congenital craniofacial disorders affecting 0.3 in 1000 livebirths worldwide. Clefts can be bilateral or unilateral and left-sided clefts occur more often. Some genetic modifiers have been associated with cleft laterality, but epigenetic contributors remain understudied.

**Methods:** We generated whole-genome DNA methylation (DNAm) data in 1 pair of monozygotic (MZ) female twins with mirror-image left cleft lip (LCL) and right cleft lip (RCL) using DNA from blood and Illumina's EPIC array. Beta values were obtained using ChAMP/RnBeads and were used to estimate the absolute DNAm differences per CpG site,  $\Delta\beta = |LCL\ \beta - RCL\ \beta|$ . Moreover, we used DNAm data from 12 unaffected females to calculate the coefficient of variation not related to clefting. After correction for cell-type heterogeneity using EpiDISH, differentially

methylated positions (DMPs) were selected based on the following criteria: (1) a coefficient of variation in DNAm levels of unaffected females < 20%, and (2) intra-twin pair absolute difference in DNAm levels >5%. Finally, we performed gene ontology (GO) and enrichment analysis.

**Results:** We identified a cluster of DMPs within 5KB upstream of the *ZFP57* gene in which the LCL MZ-twin showed significantly higher methylation levels compared to the RCL MZ-twin. We replicated this finding in an independent cohort of 13 LCL and 9 RCL cases ( $p=0.01$ ). Ontology analysis showed significant enrichment of genes involved in genomic imprinting and embryonic development, some of which contribute to left-right axis patterning.

**Conclusion:** Using mirror-image twins we identified DMPs contributing to cleft lip laterality. *ZFP57* is involved in maintenance of genomic imprinting and loss of *Zfp57* results in malformation of cardiac left and right chambers in mice, supporting its role in left-right patterning. As far as we know, this is a premier study of the association between differential DNAm and cleft laterality in nsCL.

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## 8. Developing Novel Tools to Investigate Neural Crest Cell Fate Decisions



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**Objective:** Neural crest cells (NCCs) are multipotent stem cells integral

to the formation of diverse cell types and tissues throughout the craniofacial region. Examples of NC derivatives include craniofacial bone, cartilage, and odontoblasts. Signaling pathway cues are essential for directing NC migration and fate choice decisions during development. NCC development and differentiation abnormalities result in a class of disorders known as neurocristopathies. Recent studies found distal NC enhancers and an osteoblast enhancer near *RUNX2*. Using these findings, the objective of our study was to test and validate candidate enhancer-reporters using in-vitro human NC differentiation assay, with the overall goal of designing fluorescent enhancer-reporters to track NC development and differentiation to derivatives including bone-producing osteoblasts.

**Methods:** Constructs were made using candidate enhancer sequences identified in prior studies that were active in enhancer-reporter assays in NCCs. The constructs were packaged into lentivirus, concentrated, then used to transduce iPSCs. Antibiotic selection was performed to select for stably transduced cells prior to NC differentiation where we monitored, imaged, and tracked transduced cells at various stages of NC differentiation for reporter activity. NC and osteoblast cell fates were confirmed via immunostaining.

**Results:** NCC identity from iPSC differentiation was confirmed via AP2 $\alpha$  and SOX9 marker staining. Similarly, alkaline phosphatase, Sp7, and *RUNX2* marker immunostaining confirmed osteoblast differentiation. We were then able to identify enhancer-reporters active in migratory NC (*SOX9* enhancer construct) and osteoblasts (*RUNX2* enhancer construct).

**Conclusion:** Our NC differentiation methods are adequate for obtaining migratory NC and derivatives. Additionally, the *SOX9* and *RUNX2* enhancers provide positive preliminary results for fluorescent signaling during their respective NC development stages. Dual

## Oral Session 2

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



**Brennan R. Alford**<sup>1</sup>, D.V. Wright<sup>1</sup>, C.E. Curtis<sup>1</sup>, F. Qian<sup>1</sup>, M.A. Callan<sup>1</sup>, S.D. Marshall<sup>1</sup>, T.E. Southard<sup>1</sup>, K. Shin<sup>1</sup>

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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), which applies expanding forces on the maxilla via the turning of a jackscrew of the RME, with the goal of separating the midpalatal suture. Few studies have attempted to measure these forces. The objective of this study is to pilot a novel method for measuring these forces.

**Methods:** Patients undergoing rapid maxillary expansion with 2 or 4-banded Hyrax were recruited for the study. Each expander underwent pre-clinical calibration and was delivered to each patient. Patients returned daily for 2 jackscrew turns over 15 days. The force of each turn was measured and recorded. Photos were taken and diastema width was measured during each visit. Intraoral scans were taken at the beginning and a scan and maxillary occlusal radiograph were taken after 15 days 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 forces following sutural separation, indicating that resistance to expansion is not due to the midpalatal suture alone. Older patients exhibited higher overall expansion loads with greater rates of load increase each day prior to sutural separation but exhibited similar rates of load increase as younger patients after sutural separation. Data showed a higher skeletal width increase in 4-banded hyrax expanders compared to 2-banded expanders.

reporter design is currently in progress to develop a system that can fluorescently track both migratory NCCs and osteoblasts in cell culture. With this novel tool, we hope to identify mechanisms regulating NC differentiation and candidate targets for improved bone regeneration.

Supported by: NIDCR Summer Dental Student Award program. The University of Iowa College of Dentistry Student Research Program.

**Conclusion:** Older patients exhibited 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.

Supported by: Faculty Start-up Fund.

### 10. Differential Gene and Transcript Expression in E18.5 Murine Maxillo-Mandibular Complex



**Melissa A. Morawski**<sup>1</sup>, S.L. Eliason<sup>1</sup>, S.D. Wuebker<sup>1</sup>, E. Van Otterloo<sup>1</sup>, H. Cao<sup>1</sup>, L.M. Moreno Uribe<sup>1</sup>, B.A. Amendt<sup>1</sup>, S. Rengasamy Venugopalan<sup>1</sup>

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**Objective:** The biological basis of facial asymmetry, seen in routine orthodontic patients and in congenital defects, is poorly understood. We hypothesized that there must be differential gene expression between right and left facial complex, which when perturbed will result in mild to severe facial asymmetry. The purpose of this study is to investigate differential genes/transcripts expression in the wild-type embryonic day (E) 18.5 murine right and left maxilla-mandibular (MxMn) complex.

**Methods:** Three (n=3) E18.5 C57BL/6 murine embryos were harvested and the right and left MxMn complex were hemi-sectioned in the mid-sagittal plane. We isolated total RNA using Qiazol mRNAeasy kit and confirmed equal expression of housekeeping genes by quantitative RT-PCR. Then, we performed paired-end whole transcriptome sequencing in LC Sciences (Houston, TX). We analyzed differentially expressed genes/transcripts showing >1 or <-1 log fold change ( $q < 0.05$ ). The Mouse Genome Informatics (MGI) database and gnomAD constraint scores were used to prioritize differentially genes/expressed transcripts.

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**Results:** As hypothesized, we identified 1 upregulated gene, 2 downregulated genes, 8 upregulated transcripts, and 18 downregulated transcripts in the murine MxMn complex ( $q < 0.05$ ). These genes/transcripts were associated with known craniofacial phenotypes in mouse and in humans. Most commonly affected structures were cranium, maxilla, mandible, and ear. Additionally, these differentially expressed genes/transcripts have significant gnomAD constraint scores suggesting these genes/transcripts are critical to the ontogeny of human development: LOF score  $\leq 0.31$  included 4 upregulated and 7 downregulated transcripts; LOF scores 0.31- 0.60 include 1 upregulated and 3 downregulated transcripts. Transcripts with missense Z score  $> 3.0$  include 2 upregulated and 4 downregulated transcripts.

**Conclusion:** Our findings indicate that there is a significant differential gene/transcript expression in the E18.5 murine right and left MxMn complexes. All differentially expressed genes/transcripts are critical to murine and human craniofacial development. Future studies should investigate murine models with facial asymmetry to validate our findings.

Supported by: AAOF Funding.

## 11. In-Vitro Assessment of Utilizing Torque to Confirm Bi-Cortical TAD Engagement



**Emma Mueldener**<sup>1</sup>, E. Zeng<sup>1</sup>, T.E. Southard<sup>1</sup>, S.D. Marshall<sup>1</sup>, K. Shin<sup>1</sup>

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**Objective:** Temporary anchorage devices (TADs), commonly referred to as orthodontic miniscrews, are utilized in treatment as a source of anchorage to support tooth movement. TADs are capable of engaging one bony cortex, monocortical anchorage, or two bony cortices, bicortical anchorage in the bone of the maxilla or mandible. The purpose of this study is to investigate if detection of bicortical TAD engagement in cadaver specimens utilizing torque as a metric for alternative to patient radiographic exposure.

**Methods:** Within this *in vitro* study, interrader maxillary and mandibular sites ( $n=26$ ) were engaged bicortically within two donated cadaver maxillae and mandibles. Peak insertion torque per turn was measured until TADs were fully engaged with bicortical placement, and CBCT was utilized in addition to visual reference to confirm perforation of both bony cortices. Values of peak torque per single 360° turn ( $N \cdot \text{cm} / \text{turn}$ ) and differences in torque measured between individual 360° turns were incorporated into a line-graph format then submitted for analysis of similarity with a yet to be defined via a nonparametric approach.

**Results:** Preliminary line graph distributions analyzing values of peak torque indicate a sigmoid pattern torque with progressive turns of TAD insertion, with the maximum value of measured torque corresponding to bicortical engagement. Differences in torque measured

between individual 360° turns were graphically represented and demonstrated a bimodal distribution, with peaks in difference in torque noted between engagement of the first and second cortices. When turns were delayed by a period of 5 minutes, a steep drop in peak torque value per turn was observed.

**Conclusion:** We detected torque profile from bicortical placement of TADs. Statistical analysis is in progress, which will assess for statistical significance in the torque/turn pattern of experimental sites, confirming that torque may be utilized as a metric for confirming bicortical TAD engagement.

Supported by: Faculty Start-Up Fund.

## 12. Differential Gene Expression in E11.5 Murine Right/Left Facial Prominences



**Amama Nasir**<sup>1</sup>, S.D. Wuebker<sup>1</sup>, E. Van Otterloo<sup>1</sup>, S. Eliason<sup>1</sup>, H. Cao<sup>1</sup>, L.M. Moreno Uribe<sup>1</sup>, B.A. Amendt<sup>1</sup>, S. Rengasamy Venugopalan<sup>1</sup>

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**Objective:** It is well known that, at the time of gastrulation, the formation of primitive streak establishes the right-left and anterior-posterior axes by asymmetric expression of genes. We do not know whether such asymmetric gene expression exist in the craniofacial complex, particularly at the time of facial development. The objective of this study is to investigate differential gene expression in murine right and left facial prominences in embryonic (E) day 11.5.

**Methods:** Three groups of C57BL/6 mice, with each group containing ~6-7 animals, were used in this study and the embryos were collected at day E11.5. The right and left facial prominences were carefully hemi-sectioned in the midsagittal plane and total RNA was isolated. We confirmed the quality of RNA using agarose gel electrophoresis and confirmed equal expression of house-keeping genes in both right/left facial prominences using qRT-PCR. Then, we sequenced the whole transcriptome in LC Sciences (Houston, TX). We analyzed differentially expressed genes/transcripts ( $>1$  or  $<-1$  log-fold change;  $p < 0.05$ ). Mouse Genome Informatics (MGI) database and gnomAD constraint scores were used to prioritize differentially expressed genes/transcripts.

**Results:** Comparing the right facial prominence to the left, there were 265 upregulated/ 270 downregulated transcripts and 24 upregulated/ 20 downregulated genes. Of these, 22 upregulated/ 25 downregulated transcripts and 2 upregulated/ 0 downregulated genes were reported to be associated with craniofacial phenotype in MGI database ( $p < 0.05$ ). The differentially expressed transcripts that were associated with craniofacial phenotypes also had significant gnomAD constraint scores, suggesting that the differentially expressed transcripts are critical for the ontogeny of human development.

**Conclusion:** These findings signify that there is differentially expressed transcripts between E11.5 murine right and left facial prominences. Further research in murine model with facial asymmetry is necessary to validate the findings presented.

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

## 13. Whole Genome Sequencing of a Family of Autosomal Dominant Macrostomia



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**Objective:** Most cases within the oculoauriculovertebral spectrum (OAV) are believed to be sporadic, with only one non sporadic origin recorded in a patient with Goldenhar syndrome (OAV). We present the genetic findings of a three-generation family with multiple members affected with macrosomia, preauricular tags and external ophthalmoplegia. This is, to our knowledge, the first reported autosomal dominant instance of this type.

**Methods:** We generated WGS data (30x coverage) for the proband (+OAV), affected father (+OAV) and paternal grandmother (-OAV). Genomic sequences were aligned to the human reference genome build 38 for variant calling and annotation. For variant pathogenicity we used the Mouse Genomics Informatics Database to identify genes associated with craniofacial phenotypes; gNOMAD to exclude common loss of function (LOF) or missense mutations; and VarSome to assess overall pathogenic potential. We prioritized mutations shared by the proband and father but absent in the grandmother. Finally, we used the Domino database to assess the probabilities of the mutations of interest to be autosomal dominant.

**Results:** We selected five genes, KCND2, PDGFRA, NCOA3, CASP9, WNT10A, as the most likely to harbor the etiological autosomal dominant variant. Mutations in each of them are being tested for segregation among the remaining available family members.

**Conclusion:** Our study reports the genomic findings of a unique large pedigree with multiple individuals affected with OAV spectrum phenotypes and likely autosomal dominant inheritance. Our findings narrow down to five potential candidate genes, KCND2 having high pathogenic potential and very likely dominant inheritance. WNT10A and CASP9 are known craniofacial genes, while PDGFRA and NCOA3 show very likely dominant inheritance. Identifying an autosomal dominant gene specifically causing macrostomia would not only give vital information to families experiencing generational macrostomia but be a foundation for

researchers to further study the etiological factors leading to the condition.

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## 14. TFAP2 Indirectly Regulates Odontogenesis by Promoting a Skin Epithelial Program



**Carter Coppinger**<sup>1</sup>, J. Thompson<sup>1</sup>, F. Shao<sup>1</sup>, H. Cao<sup>1</sup>, E. Van Otterloo<sup>1</sup>

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**Objective:** The pathological gain, loss, or misplacement of teeth can occur in a variety of syndromic or non-syndromic congenital anomalies and can profoundly impact those afflicted. An understanding of the early control of tooth initiation will provide inroads for predicting and ameliorating these defects. The hallmark sign of tooth initiation is the formation of a thickened strip in the oral epithelium, known as the dental lamina (DL). While numerous mouse models have pinpointed genes controlling tooth development, the majority do not impact initial DL formation. We identified that early (prior to DL formation) ectodermal loss of two TFAP2 paralogs, TFAP2A and TFAP2B, is associated with duplicated incisors, although the mechanism is 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 TFAP2ECT mutants, we predict there is an expansion of the oral epithelial program, concomitant with an expanded DL, leading to ectopic incisors. To test this hypothesis, we use conditional mouse genetics to delete both *Tfap2a* and *Tfap2b* in the ectoderm collect embryos at key embryonic stages for further analysis. Collected 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; and how these changes are impacting the dental mesenchyme.

**Results:** In situ hybridization and single cell RNA-sequencing suggests an expansion of the oral/DL epithelium, at the expense of the surface ectoderm, in TFAP2ECT mutants, relative to controls. Further, epithelial changes in gene expression resulted in concomitant changes in the underlying mesenchyme, including an expansion of the odontogenic related gene, *Msx1*.

**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: The University of Iowa College of Dentistry Student Research Program.

## 15. DNA Methylation and Van Der Woude Syndrome Phenotypic Variability



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**Objective:** Van der Woude Syndrome (VWS) is an autosomal dominant disorder and the most common syndromic form of orofacial clefting. VWS is caused by mutations in *IRF6* (70%) and *GRHL3* (5%) and classically presents with combinations of lip pits (LP), cleft lip (CL), cleft lip and palate (CLP), or cleft palate (CPO). The markedly phenotypic discordance even amongst individuals that carry the same mutation suggests a possible role for epigenetic factors as phenotypic modifiers. *IRF6* and *TP63* interact in a regulatory loop that coordinates epithelial proliferation and differentiation in palatogenesis. We hypothesize that differential DNA methylation (DNAm) in CpG sites within regulatory regions of *IRF6* and *TP63* are associated with VWS phenotypic discordance.

**Methods:** We measured DNAm levels of CpG sites located in the promoters of *IRF6* and *TP63*, and in a known *IRF6* enhancer element in 83 individuals with *IRF6*-associated VWS. Blood DNA samples were bisulfite converted and pyrosequenced with primers specific to the target regions. We compared the methylation levels of each CpG amongst 5 phenotype groups (1=CL/P+LP, 2=CL/P, 3=CPO+LP, 4=CPO, 5=LP) using one-way ANOVA on ranks with the post-hoc Tukey-Kramer test ( $\alpha=0.05$ ).

**Results:** CpG sites in the promoter region of *IRF6* showed statistically significant differences in methylation ( $P<0.05$ ). Individuals with any form of cleft (groups 1-4) had significantly higher mean/median methylation levels than individuals with lip pits only (group 5), while no difference was found when comparing phenotype groups with a cleft (groups 1-4). We found no significant differences in methylation in the *TP63* promoter and *IRF6* enhancer among the studied phenotypic groups.

**Conclusion:** Our results indicated that hypermethylation of the *IRF6* promoter (in addition to the causal mutation) is associated with more severe VWS phenotypes (any cleft +/- lip pits); thus, possibly impacting an already impacted *IRF6* protein due to a genetic mutation and leading to a more severe phenotype.

Supported by: NIH/NIDCR K01DE027995. The University of Iowa College of Dentistry Student Research Program.

## 16. Cross-Repression of Pitx2 and Tfp2a/Tfp2b Regulating Tooth Initiation



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**Objective:** In human and mouse, tooth morphogenesis begins with dental lamina (DL) formation. Our preliminary study shows that there is distinct spatial patterning of transcription factors (TFs) expression along mandibular epithelium. Specifically, TFs including *Pitx1*, *Pitx2*, *Sox2* are enriched in lingual ectoderm and DL, while *Tfp2a* and *Tfp2b* are enriched in labial and aboral ectoderm. However, whether there is an interaction between *Pitx2* and *Tfp2a/Tfp2b* in regulating tooth initiation is unknown.

**Methods:** *Pitx2* general knockout (*Pitx2*<sup>null</sup>) and *Tfp2a/Tfp2b* ectodermal double knockout (*Tfp2a/b*<sup>edko</sup>) mouse models were employed in this study. DL of *Pitx2*<sup>null</sup> embryos and aboral epithelium of *Tfp2a/b*<sup>edko</sup> embryos were collected for RNA-seq. Dysregulated genes were analyzed comparing with their littermates and validated by immunofluorescence (IF) staining. The interaction between *Pitx2* and *Tfp2a/Tfp2b* was assessed in GSMK and 293T cells for *in vitro* validation.

**Results:** RNA-Seq data and IF staining revealed that *Tfp2a* and *Tfp2b* were upregulated in *Pitx2*<sup>null</sup> DL, while *Pitx2* and *Sox2* were upregulated in *Tfp2a/b*<sup>edko</sup> aboral epithelium. *In vitro* results showed *Tfp2a* mRNA expression and protein level were decreased in *Pitx2*-overexpressed GSMK cells. In addition, TFAP2 can inhibit PITX2 activation activity.

**Conclusion:** We identified a novel transcription factor interaction that cross-repression between *Pitx2* and *Tfp2a/Tfp2b* is involved in regulating dental lamina morphogenesis. However, the molecular mechanism needs further investigation.

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## Oral Session 3

### 17. A Survey on Restoring Endodontic Access Openings Through All-Ceramic Restorations



**Connor A. Dang**<sup>1</sup>, R. Bowers<sup>1</sup>, C. Barwacz<sup>1</sup>, F. Qian<sup>1</sup>, C. Hogden<sup>1</sup>

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**Objective:** A plethora of ceramic biomaterials are currently available to the practicing dentist. Clinical management of these all-ceramic restorations varies by material. Restoring endodontic access openings through all-ceramic restorations using inadequate bonding protocols may contribute to coronal leakage which might compromise endodontic treatment outcomes. The aim of this study was to investigate the knowledge, attitudes, training, and practice of active members of the American Association of Endodontists (AAE) regarding restoring access openings through all-ceramic restorations.

**Methods:** A cross-sectional survey study involving 30 multiple-choice questions was constructed and distributed electronically using Qualtrics to active members of the AAE (n=3962). The anonymous survey was available for one month with a reminder email sent two weeks after the initial invitation. Statistical analysis consisted of descriptive, univariable, and multivariable logistic regression analyses ( $\alpha=0.05$ ).

**Results:** A total of 351 subjects (79.5% male; 42% 50+ years old) completed the survey with a 9.1% response rate. Nearly 90% of respondents restore access preparations through all-ceramic restorations. Resin-based composite was the most frequently used material (87.9%) with 35.6% of composite users reporting use of a bonding protocol specific to the type of all-ceramic material. The final multivariable logistic regression model revealed that respondents who received training on handling all-ceramic materials (OR=2.29;  $p=0.015$ ), verified material identity (OR=12.06;  $p<0.001$ ), and practiced in an academic setting (OR=5.28;  $p=0.002$ ; reference: owner/partner) were more likely to use material dependent bonding procedures.

**Conclusion:** Most respondents do not currently follow a bonding protocol based on material type. Practicing in academics, receiving training on management of all-ceramic materials, and verifying material identity were positively correlated with following material dependent bonding protocols.

Supported by: AAE Foundation. Department of Endodontics at Iowa.

### 18. Non-Viral Nanoparticles Delivering Plasmid DNA Encoding miR-200c for Regenerative Endodontics



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MicroRNAs (miRs) play important post-transcriptional regulators in inflammation and regeneration, and have emerged as innovative tools for the diagnosis of diseases and treatment. *miR-200c*, a member of the miR-200 family, has previously been demonstrated to potentially regulate tooth development, modulate inflammation, and promote osteogenesis, and could also be a candidate to assist in regenerative endodontics procedures.

**Objectives:** To characterize *miR-200c* in pulpitis and determine its potential in attenuating inflammation and promoting odontogenesis in dental pulp stem cells (DPSCs).

**Methods:** *miR-200c* and proinflammatory cytokine expression levels from human dental pulp diagnosed with irreversible pulpitis and healthy pulp were quantified using RNA sequencing (miRNA-seq, mRNA-seq) and PCR. Transfection efficiency of plasmid DNA (pDNA) encoding *miR-200c* to transfect human dental pulp stem cells (hDPSCs) delivered by CaCO<sub>3</sub>-based nanoparticles was also assessed. *IL-6* and *IL-8* transcript and protein levels of hDPSCs with *miR-200c* overexpression were quantified after exposure to P.gingivalis lipopolysaccharide (Pg-LPS). Odontogenic differentiation markers in hDPSCs were additionally assessed after *miR-200c* overexpression via qPCR and western blotting. Finally, a rat pulp capping model was used to evaluate the function of *miR-200c in vivo* using histology. Statistical analyses included one-way ANOVA and Student T-Tests.

**Results:** We observed that *miR-200c* was downregulated in inflamed pulps, while *IL-6* and *IL-8* were significantly upregulated (n=12). CaCO<sub>3</sub> nanoparticles effectively improved the transfection efficiency of pDNA with minimal cytotoxicity in hDPSCs. Overexpression of *miR-200c* using the CaCO<sub>3</sub> delivery system significantly increased hDPSCs odontogenic differentiation markers including OCN, DMP1 and DSPP. Additionally, *miR-200c* also effectively downregulated *IL-6* and *IL-8* under the Pg-LPS challenge in hDPSCs and mitigate inflammation in a rat model of pulpitis.

**Conclusion:** Our findings demonstrated that pDNA encoding *miR-200c* delivered by CaCO<sub>3</sub> nanoparticles might serve as a novel therapeutic agent for regenerative endodontics by attenuating inflammation and promoting odontogenic differentiation.

Supported by: NIDCR R01 DE02643. Department of Endodontics at Iowa.

## 19. Retrospective Study of Endodontic Outcomes Treated by Junior Students



**Alexis L. Borgestad<sup>1</sup>, M.R. Gomez<sup>1</sup>, R.E. Walton<sup>1</sup>**

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**Objective:** The aim was to radiographically evaluate initial non-surgical treatment outcomes retrospectively. Hybrid Rotary NiTi

(reciprocal) instrumentation technique was used. Also evaluated were how clinical outcomes were affected when a procedural canal preparation error occurred.

**Methods:** Utilizing the school-based Axiom system, 700 patient records with an endodontic recall code were accessed from 2013 through 2018. The qualifying sample was 53 root-filled teeth that had NSRCT performed by a junior dental student followed by at least a 1-year recall. Two board certified endodontists reviewed treatment digital periapical radiographs in a controlled setting. Evaluated were 1) errors performed during treatment and 2) treatment outcome on recall. Outcomes were categorized as 1. favorable prognosis, 2. questionable prognosis, 3. unfavorable prognosis. Descriptive analysis was performed due to low sample size.

**Results:** 53 initial root canal treatment cases were completed: 22 anteriors, 19 premolars, and 12 molars. Outcome prognosis overall 89% favorable (47/53), 0% questionable (0/53), and 11% unfavorable (6/53). Errors occurred in 32% (17/53), 5% in anteriors (1/22), 37% in premolars (7/19), and 75% in molars (9/12). Outcome prognosis for teeth with an error performed was 76% favorable (13/17) and 24% unfavorable (4/17).

**Conclusion:** Within the limitations of this study, overall prognosis for Hybrid Rotary NiTi (reciprocal) instrumentation completed by junior dental students was comparable to historic success rates. Posterior teeth were more prone to have procedural errors and an unfavorable prognosis. When an error occurred outcome prognosis decreased about 10%.

## 20. Articaine Use and Practitioner-Associated Trends – United States Survey Investigation



**Olivia C. Bartholomew<sup>1</sup>, F. Qian<sup>1</sup>, C. Hogden<sup>1</sup>, B. Howe<sup>1</sup>, R. Bowers<sup>1</sup>**

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**Objective:** Local anesthesia is an essential component of clinical dental practice, but there is a limited quantifiable understanding of what techniques and

local anesthetic solutions are utilized by practicing dentists in the United States (US). Articaine, a dental local anesthetic, has been highly debated regarding its efficacy and risks for toxicity or paresthesia. The objective was to investigate the extent of articaine

use by US dentists and the associated determinate factors related to practitioner reports. The specific aims highlight potential future initiatives in dentistry to improve the calibration of pain management and safety practices.

**Methods:** A 24-item questionnaire regarding demographics and local anesthesia practices, including articaine-specific items, was sent via email or mail to 11,600 practicing general dentists and specialists throughout the US. Statistical analysis consisted of descriptive, bivariate, and multivariate logistic regression.

**Results:** A total of 1,128 subjects (69.2% males, 49.4% general dentists) completed the survey with a response rate of 9.7%. Previous experience with articaine was reported by 97.6% of respondents (3.3% of which reported no longer using articaine currently). Nearly 37% of respondents reported using articaine for all injections involving vasoconstrictors. The data showed respondents were more likely to be male (OR=1.43; p=0.013), general dentists (OR=1.51; p=0.003), in clinical practice for >20 years (OR=1.33; p=0.028), and practice in rural communities (OR=1.57; p=0.016). Fifty-five percent of respondents reported concerns of paresthesia risk related to articaine use, while 14% of respondents reported concerns of overdose toxicity.

**Conclusion:** The results indicated a notable variation in the application and frequency of articaine use among US practitioners. Expanding on non-biased, well-designed investigations concerning articaine's efficacy and safety will help improve the standardization of local anesthesia practices in the future.

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

## 21. Effectiveness of SDF on Caries Lesions in Adults: Retrospective Study



**Ala Al Saffer<sup>1</sup>, J.L. Kolker<sup>1</sup>, S. Guzman-Armstrong<sup>1</sup>, E. Zeng<sup>1</sup>, L. Marchini<sup>4</sup>, A. Elgreatly<sup>2</sup>**

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**Objective:** Silver diamine fluoride (SDF) is used to treat dental caries lesions on primary teeth in children and have been found effective. Older adults may also benefit from the use of SDF in treating exposed initial root caries lesions, yet such studies are lacking. The aim of this retrospective study was to determine the factors related to longevity of adult patients' teeth after SDF treatment.

**Methods:** Data from the University of Iowa College of Dentistry data base (AxiUm) were used for this study. Patients aged 18 years and older with one or more code D1354 between 2016 and 2021 were included. Patient variables included: age, gender, ethnicity, number

of medications, smoking status, radiation therapy, insurance status, and number of D1354 codes overall. Tooth variables included: tooth number, number of D1354 codes applied on the tooth, and subsequent treatment (restoration, root canal treatment, extraction). Operator variables included: provider type (student, post-grad, faculty) and type of clinic (geriatric vs. other). The dependent outcome variable was survival defined as not needing subsequent treatment. Statistical software was used to calculate chi-square values used for analyses to determine relationship between variables and survival.

**Results:** Results included 1772 patients with 2985 SDF treated teeth. Patients that had significantly higher failure (p <.001 to <.05) were aged 75+, white, had private insurance, had higher number of medications, severe dry mouth, tobacco use, and breathing or lung problems.

**Conclusion:** After SDF, older white patients with private insurance and indicators of dry mouth had higher failure rates.

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## 22. Influence of Print Directions on Properties of 3D Printing Resin



**Josephine Itakpe<sup>1</sup>, A. Tsujimoto<sup>1</sup>, E. Teixeira<sup>1</sup>, F. Qian<sup>1</sup>, C. De Mattos Pimenta Vidal<sup>1</sup>, A. Mahrous<sup>2</sup>**

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**Objective:** To assess influence of print direction and orientation on mechanical properties and dimensional accuracy of 3D printable resin-based composites.

**Methods:** Permanent Crown Resin (Formlabs) and Temporary CB Resin (Formlabs) were used to print specimens in (0o, 45o, and 90o) orientations using a Desktop 3D Printer (Form 2, Formlabs). Ninety-six specimens (2mmx2x25mm) were printed to assess dimensional accuracy and flexural strength. seventy-two cylindrical-shaped specimens (6mmx3mm) were for localized wear testing. A digital micrometer (ABSOLUTE Digimatic, Mitutoyo) was used to measure length, width, and height of specimens (n=16). Flexural strength testing was conducted (n=16) in three-point bending following ISO 4049. While localized wear testing (n=12) was stimulated using 400,000 cycles in a Lenifelder-Suzuki (Alabama) machine. Statistical analysis included two-sample t-test, one- and two-way ANOVA, post-hoc Tukey's test Wilcoxon rank-sum test, Kruskal-Wallis test, and Friedman's two-way nonparametric ANOVA when appropriate (alpha=0.05).

**Results:** Mean length and width dimensions were significantly higher in the 0° direction for permanent resin compared to temporary resin (p<0.001). For both materials, mean height at 0° direction was significantly

higher than those observed in 90° and 45° directions (p<0.001), while no difference was noted between 90° and 45°. Permanent resins showed significantly higher flexural strength than temporary resin in all print directions (P<0.001). mean maximum depth and volume loss of permanent resin was significantly lower than that of temporary resin (p<0.05). Significant interactions between resin type and print direction on width or height dimension were noted (p<0.05).

**Conclusion:** Dimensional accuracy and mechanical properties were influenced by a change in the print direction and orientation in the permanent and temporary crown resin.

## 23. Sintering Effect on Zirconia Manufactured by Suspension Enclosed Projection Stereolithography



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**Objective:** To evaluate the suitability of using a zirconia dental material with a novel 3D printing process named suspension enclosed projection stereolithography (SEPS); and to investigate the effect of sintering time and temperature on the flexural strength and density of zirconia produced by SEPS.

**Methods:** A slurry composed of 60% 3mol% yttria-stabilized zirconia (YTZP- Zpex, Tosoh), 40% 3D printing resin (20% ANYCUBIC white 405nm UV resin, 20% Formlabs Clear Resin), and 1.5% Solsperse 20000 dispersant, 0.5% Isopropyl Alcohol was prepared, ball milled, and degassed. Fifty bar specimens were fabricated via SEPS manufacturing (Curing Time — 45 seconds/layer. 0.062mm layer thickness, 28 layers) and post-cured under UV light for 10min. Specimens were debinded at 600°C for 180min. Samples were categorized into 4 groups (n=10 per group): A:1,450°C for 120min, B:1,530°C for 120min, C:1,530°C for 30min, and D:1,530°C for 10min, with a heating rate of 8°C/min using a Ceramill Therm 3 sintering furnace. Sintered specimens (21mm x 3mm x 0.9mm) were measured with a digital micrometer. Flexural strength was tested in three-point bending at crosshead speed of 1 mm/min. Density was measured by the Archimedes method (n=5) and phase composition was determined by X-Ray diffraction.

**Results:** One-way Welch ANOVA showed that there was no significant difference in mean flexural strength among the groups (p=0.362). Kruskal-Wallis followed by Dunn's test indicated a significant difference for density between groups B and D (p=0.0326). X-ray diffraction data confirmed the presence of predominantly tetragonal phase.

Continued ►

**Conclusion:** 3Y Zirconia printed via SEPS manufacturing can be sintered at a higher temperature with shorter dwell times compared to manufacturer recommendations for milled zirconia materials. X-ray diffraction and density data confirmed that different settings can be used to fully sinter 3D printed zirconia for potential dental applications.

## 24. Analysis of Novel Streptococcal Probiotic Candidates' Antimicrobial and Anti-Inflammatory Properties



**Samuel L. Allen**<sup>1</sup>, J.A. Banas<sup>1</sup>, M. Zhu<sup>1</sup>  
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**Objective:** The oral microbiome consists of intricate interactions between organisms that when out of balance can be a primary factor in the development of oral disease. An option for reducing the risk of oral disease is to identify probiotic strains that inhibit the growth of oral pathogens or in some way promote oral health. We hypothesize that bacterial strains isolated from children with a caries free history will be a source of probiotic candidates that inhibit the growth of cariogenic or periodontal pathogens or suppress chronic inflammation.

**Methods:** Antagonistic effects of candidate probiotic strains were assessed by measuring zones of inhibition when grown in close proximity to pathogenic (*S. mutans*, *S. sobrinus*, *C. albicans*) or health-related (*S. sanguinis*) oral microbes. Quantitative Polymerase Chain Reaction was used to quantify relative expression of pro- and anti-inflammatory cytokines in co-cultures of probiotic candidates and mouse macrophage RAW 264.7 cells.

**Results:** Two (HP3 and HP5) of ten mildly acidogenic probiotic candidates were found to possess strong antagonistic activity towards *S. mutans*. Four (IOWA1-4) other probiotic candidates, previously determined to inhibit *S. mutans*, significantly elevated expression of the anti-inflammatory cytokine IL-10 in a mouse macrophage co-culture.

**Conclusion:** The data from the candidates' antagonism of *S. mutans* and influence on cytokine expression supports the hypothesis, but the effectiveness and limitations of each candidate strain vary suggesting that the most effective approach to promote oral health via probiotics will be a combination of strains with diverse properties.

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

## Oral Session 4

## 25. Relative Contributions of Diet in Shaping the Oral Microbiome



**Arshi Munjal**<sup>1</sup>, A. Sidahmed<sup>1</sup>, K. Pax<sup>25</sup>, S.M. Dabdoub<sup>1</sup>, P.S. Kumar<sup>25</sup>, S.M. Ganesan<sup>1</sup>

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**Objective:** It is well known that diet impacts the gut microbiome. Prior studies have demonstrated that plant-based vs meat-based diets alter the composition and function of the gut microbiome. Although the oral cavity is the beginning of the gastrointestinal tract, the impact of diet on the oral microbiome is understudied. Therefore the goal of our study was to compare the microbial composition and co-occurrence patterns of those who consume plant-based (PB) and meat-based (MB) diets across different niches in the oral cavity.

**Methods:** Unstimulated saliva, supra and sub gingival plaque samples were collected from 24 frequency matched, periodontally and systemically healthy volunteers belonging to two groups: 1) plant-based diet (H-PB) and meat-based diet (H-MB). DNA was isolated, 16S sequencing performed (Illumina MiSeq V1-V3, V7-V9 region) and annotated against the SILVA Database.

Distant metrics were calculated, Principal component analysis (PCA) performed on variance-stabilized ASV counts, and significance was determined using Adonis with 999 permutations. Tukey HSD and DESeq2 were used to determine statistical significance.

**Results:** Alpha and beta diversity did not differ significantly between the H-PB and H-MB groups in any of the niches of the oral cavity. Statistical analysis revealed no significant differences at the phyla and genera level in any niches, except at the supragingival microbiome of H-MB group, which exhibited higher abundances of *Aggregatibacter* than H-PB. Functional analysis mirrored the compositional patterns, and no significant functional pathways differed between the diet groups in salivary environment. Functions related to transcription factors were increased in supragingival environments of H-MB microbiome.

**Conclusion:** Microbial composition and predicted functions between the two diet groups in subgingival, supragingival, and salivary environments were mostly similar. Thus, our preliminary investigation suggests that when patients are frequency-matched for age, gender, ethnicity, and are controlled for external factors, the impact of diet on the oral microbiome is minimal.

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

## 26. Therapeutic Potential of 6-hydroxycaproic Acid: A Metabolite of *Streptococcus gordonii*



**Chawin Upara**<sup>176</sup>, Q. Ding<sup>1</sup>, M. Zhu<sup>1</sup>, J.A. Banas<sup>1</sup>, L. Hong<sup>1</sup>

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**Objective:** We have previously demonstrated that the spent culture supernatant of *Streptococcus gordonii* (Sg-SCS) suppresses the growth and attachment of the keystone periodontal pathogen *Prophyromonas gingivalis*, as well as the red complex *Treponema denticola*. Sg-SCS also reduces IL-1 $\beta$ , IL-6, and IL-8 transcript and protein levels in cells challenged with *P. gingivalis* lipopolysaccharide (Pg-LPS). These results indicate that certain metabolites in Sg-SCS may be candidate biologics to suppress the bacteria and inflammation that accompany periodontal disease. In this study, we aim to identify the specific metabolites responsible for those anti-bacterial and anti-inflammatory effects using *in vitro* cell culture modules to develop a therapeutic treatment for periodontitis.

**Methods:** 6-hydroxycaproic acid (HCA), a medium chain fatty acid, is a metabolite that was found to be significantly increased in Sg-SCS using untargeted metabolomics. The effects of HCA on periodontal bacterial growth and pro-inflammatory cytokine expression in Pg-LPS-challenged human periodontal and immune cells were investigated through use of *in vitro* cell culture studies using HCA-incorporated culture medium. MTT assays were also performed to evaluate the effect of HCA on cellular toxicity.

**Results:** HCA presents no toxicity to human gingival fibroblasts and monocyte-derived macrophages. Similar to Sg-SCS, HCA reduced the growth of *T. denticola* and *Streptococcus oralis*. HCA also reduced the expression of *IL-1b*, *IL-6*, and *IL-8* in human gingival fibroblasts and monocyte-derived macrophages challenged with Pg-LPS.

**Conclusion:** HCA suppresses the growth of pathogenic periodontal bacteria and demonstrates anti-inflammatory effects with no cellular toxicity, indicating its therapeutic potential for treating periodontal disease.

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## 27. IL-1 Receptor Antagonist Plays a Protective Role In Ligature-Induced Periodontitis



**Jinmei Zhang**<sup>1</sup>, A.X. Wang<sup>1</sup>, Z. Wang<sup>1</sup>, S. Hongli<sup>1</sup>, S. Zhang<sup>1</sup>

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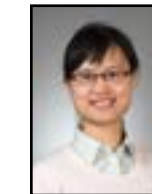
**Objective:** We aimed to assess the role of interleukin -1 receptor antagonist (IL-1RA) in ligature-induced periodontitis (LIP) and the mechanism of IL-1RA in periodontitis bone loss.

**Methods:** Periodontal bone loss was induced through the LIP model in WT and *Il1ra*<sup>-/-</sup> mice and measured by micro( $\mu$ ) CT. Transcription of genes associated in inflammatory pathways was compared by the real-time-quantitative PCR (RT-PCR) between WT and *Il1ra*<sup>-/-</sup> ligated gingivae. Single-cell suspensions were prepared in gingiva and cervical lymph nodes and were analyzed by fluorescence-activated cell sorting to quantify IL-17+ cells and IL-17-secreting subpopulations. We locally delivered IL-1RA-complexed nanoclay, a controlled drug releasing system, in the ligated gingiva and compared the bone loss with WT mice that received control treatment.

**Results:** *Il1ra*<sup>-/-</sup> mice demonstrated significant bone loss as compared to WT mice in the LIP model. Expression of *Il1b*, *Il6*, *Il23*, *Tgfb*, *Il17*, *Inos*, *Mrc1*, *Mmp13*, and *Rank* was significantly upregulated in the ligated gingiva of *Il1ra*<sup>-/-</sup> mice than WT mice. Significantly more IL-17+ immune cells (CD45+IL17+) are present in the gingiva of *Il1ra*<sup>-/-</sup> mice with the majority of being TCR  $\gamma\delta$  T cells (CD45+IL-17+CD3+TCR  $\gamma\delta$ +) than WT mice. The IL-1RA-nanoclay treatment significantly attenuated the alveolar bone loss than the control-treated mice in the LIP model (0.29  $\pm$  0.06 versus 0.39  $\pm$  0.08, p=0.03).

**Conclusion:** IL-1RA plays a protective role in the murine ligature-induced model by suppressing IL-17 responses

## 28. Vanillin-Bioglass Cross-linked 3D Chitosan Scaffolds for Bone Regeneration



**Jue Hu**<sup>1</sup>, Z. Wang<sup>1</sup>, J. Miszuk<sup>1</sup>, J. Faber<sup>1</sup>, H. Sun<sup>1</sup>

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**Objective:** Chitosan is an inexpensive biodegradable natural polymer for tissue engineering. However, the clinical application of chitosan in bone repair is impeded due to its poor mechanical properties. We recently developed a new vanillin-bioglass based crosslinking technique to generate an innovative antibacterial three-dimensional (3D) porous chitosan-vanillin-bioglass (CVB) scaffold with improved

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mechanical properties, and osteoconductivity. We hypothesize that our new CVB scaffolds have great potential to promote stem cell osteogenic differentiation and endogenous bone regeneration through its unique physical-chemical features.

**Methods:** The 3D CVB scaffolds were prepared by freezing the CVB hydrogel followed by lyophilization. Gelation time was controlled within a few minutes. Cell proliferation and differentiation were quantified using MTS assay, ALP activity test, Alizarin Red S staining and RT-PCR. In addition to the pro-osteogenic abilities, CVB scaffolds also significantly reduce the LPS-stimulated inflammatory cytokines with strong anti-inflammatory properties. To further improve the drug delivery capacity, we decorated the CVB scaffolds with poly (glutamic acid) which was significantly improve the BMP2 binding and osteogenic differentiation *in vitro*. The *in vivo* bone formation ability of the CVB scaffolds was tested by an ectopic bone model in mice.

**Results:** Our data indicated that the CVB scaffolds not only had good biocompatibility, strong anti-inflammatory properties and antibacterial ability but also significantly promoted osteoblastic differentiation, mineralization *in vitro*, and bone formation *in vivo*.

**Conclusion:** Thus, our findings suggest the CVB scaffolds hold great promise for bone tissue engineering applications based on their robust mechanical properties, osteoconductivity, antibacterial, and anti-inflammation abilities.

Supported by: R01DE029159. T90DE023520.

## 29. Longitudinal Fluoride Intake and Bone Densitometry Outcomes at 23 Years



**Gurjot Kaur Bhatia**<sup>1</sup>, S.M. Levy<sup>1</sup>, C. Pendleton<sup>1</sup>, J.J. Warren<sup>1</sup>, P. Saha<sup>1</sup>, E. Zeng<sup>1</sup>

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**Objective:** Concerns have been raised about the impact of fluoride intake on young adults' developing skeletons, but gaps exist in our understanding of this relationship. This analysis assessed the relationships between period-specific and cumulative daily fluoride intakes and bone densitometry outcomes of 23-year-olds.

**Methods:** Secondary data analysis of Iowa Fluoride Study (IFS)/Iowa Bone Development Study (IBDS) data included Multi-Detector Computed Tomography (MDCT) scans of the distal tibia from 330 participants. Nine cortical and four trabecular bone microstructural outcomes were computed using previously-validated methods. Most participants lived in optimally fluoridated areas. Estimated period-specific (0-8, 8-15, 15-23 years) and cumulative (birth to 23 years) fluoride intakes from combined sources were determined. Their sex-stratified bivariate (unadjusted) associations with MDCT outcomes were examined using Spearman correlation coefficients. Due to the multiple statistical analyses

being conducted with the same explanatory fluoride variables, p-values <0.01 were considered statistically significant and those with 0.01<p<0.05 were considered statistically suggestive.

**Results:** Daily fluoride intakes were 0.73-1.12 mg F for males and 0.67-0.93 mg F for females. Most Spearman correlations between daily fluoride intake and MDCT measures were weak. Spearman correlations for females were r = -0.13-0.18 and for males they were r = -0.17-0.23. Among the 36 fluoride associations with trabecular measures, only 6 (5 for males and 1 for females) had statistically suggestive bivariate (unadjusted) correlations (p<0.05), none were statistically significant (p<0.01), and they were all positive and weak. None of the fluoride intakes had statistically significant (p<0.01) or statistically suggestive (0.01<p<0.05) correlations with cortical measures for either sex.

**Conclusion:** Longitudinal period-specific and cumulative fluoride intakes were associated weakly and mostly positively with MDCT bone measures at age 23 years. Hence, fluoride intakes within the optimal range do not appear to have any adverse impact on bone health in young adults.

Supported by: NIH M01-RR00059. NIH R01-DE09551. NIH R01- DE12101. NIH UL1-RR024979. University of Iowa Wright-Bush-Shreves Endowed Professor Fund. The Delta Dental of Iowa Foundation.

## 30. Exogenous Bone Sialoprotein Enhances Alveolar Bone Healing in Mice



**Michael B. Chavez**<sup>1</sup>, M. Tan<sup>25</sup>, T. Kolli<sup>25</sup>, E. Chu<sup>48</sup>, H.A. Goldberg<sup>69</sup>, B.L. Foster<sup>25</sup>

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

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<sup>48</sup>University of Maryland, Baltimore, MD;

<sup>69</sup>University of Western Ontario, London, ON

**Objective:** Bone sialoprotein (Ibsp/BSP) is an extracellular matrix protein associated with mineralized tissues and shown to promote hydroxyapatite nucleation and growth. BSP includes a collagen-binding motif, 2-3 polyglutamic acid hydroxyapatite-nucleating domains, and an RGD integrin-binding sequence; post-translational modifications (PTMs) including serine phosphorylation and O/N-linked glycosylations also contribute to mineralization functions. Ibsp knockout (Ibsp<sup>-/-</sup>) mice exhibit defective bone formation and remodeling, with alveolar bone showing more pronounced hypomineralization than long bones. We found ~40% reduction in alveolar bone healing in Ibsp<sup>-/-</sup> vs. wild-type (WT) control mice. We hypothesized adding exogenous BSP would rescue defective alveolar bone healing in Ibsp<sup>-/-</sup> mice.

**Methods:** First maxillary molars were bilaterally extracted from 42 days postnatal Ibsp<sup>-/-</sup> and WT mice. Collagen gel with or without BSP was delivered to sockets. BSP included native rat long bone BSP (nBSP) and recombinant rat BSP (rBSP). Bovine type I collagen

remained liquid while on ice and solidified to gel at 37°C. Tissues were harvested 14 days post-procedure and analyzed by microCT and histology.

**Results:** Bone volume fraction (BV/TV) was increased by both nBSP (53%; P<0.001) and rBSP (69%; P<0.0001), compared to collagen vehicle in Ibsp<sup>-/-</sup> mice. BMD of new bone was increased by nBSP (54%; P<0.0001) and rBSP (66%; P<0.0001) compared to collagen only in Ibsp<sup>-/-</sup> mice. Both nBSP and rBSP normalized BV/TV and BMD in Ibsp<sup>-/-</sup> mice to WT levels. Surprisingly, in WT mice, BV/TV of healing alveolar bone was also increased by nBSP (34%; P<0.001) and rBSP (65%; P<0.0001) compared to controls. BMD of new bone in WT mice was increased by nBSP (35%; P<0.0001) and rBSP (62%; P<0.0001) vs. controls.

**Conclusion:** Exogenous BSP rescued alveolar bone healing defects in Ibsp<sup>-/-</sup> mice and enhanced bone healing in WT mice. Equivalent effects of nBSP and rBSP suggest PTMs are not essential for BSP functions in bone healing.

Supported by: NIH F30DE030358-02 (MBC). NIH 5R01DE027639-04. The University of Iowa College of Dentistry Student Research Program.

## 31. Functionalize 3D-printed PCL Scaffolds with Nanoparticles for Bone Tissue Engineering



**Jessica Faber**<sup>1</sup>, H. Sun<sup>1</sup>, J. Miszuk<sup>1</sup>, J. Hu<sup>1</sup>, Z. Wang<sup>1</sup>

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

**Objective:** Repair of critical-sized bone defects remains a relevant and unmet clinical challenge. Autologous bone grafts, the gold standard for treatment, are accompanied by a host of potential complications, leading researchers to explore other treatment strategies. Biomimetic scaffolds are an essential tool for bone regeneration, and 3D printing is emerging as a promising method for fabrication. 3D-printed polycaprolactone (PCL) scaffolds possess many desirable properties including low cost, customization, and standardization. However, the conventionally printed PCL scaffolds are bioinert and lack the capacity for cell/osteogenic factor delivery while new bioprinting techniques are complicated and less translational thus far. This study aims to utilize polydopamine and nanoclay to create a bioactive, osteogenic 3D-printed scaffold. Collagen nanofibers constitute the main components of the bone matrix and are widely used alone or with other materials for cell and drug delivery. Due to the hydrophobic nature of PCL, a surface modifier is needed to facilitate collagen-PCL binding. Polydopamine nanoparticles can improve the scaffold's surface properties allowing collagen to coat the scaffold. Moreover, our recent studies indicate that nanoclay can not only promote osteogenic differentiation but can also bind drugs (proteins, e.g.,

BMP2 or small compounds, e.g., FTY720). Therefore, we use nanoclay to improve the drug delivery and osteogenic capacity of the PCL/collagen system.

**Methods:** The osteogenic potential of the proposed system was evaluated using *in vitro* cell culture of MC3T3-E1 cells. The structure and surface properties of the scaffold were assessed by scanning electron microscopy.

**Results:** Our *in vitro* results indicated that nanoclay+FTY could increase osteogenic differentiation marker ALP and enhance matrix mineralization. The results also showed polydopamine can encourage the attachment of collagen fibrous gel to the PCL scaffold to create a bioactive scaffold for bone tissue engineering.

**Conclusion:** The findings suggest that our functionalized 3D-printed scaffold with nanoparticles has great potential for bone tissue regeneration.

Supported by: NIH R01DE029159.

## 32. Fluoride Knowledge, Attitudes, and Behaviors in Green Hill, Alabama Adults



**Alex Harper**<sup>1</sup>, S.M. Levy<sup>1</sup>, W. Shi<sup>1</sup>

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**Objective:** People's knowledge, attitudes, and behaviors are important determinants of oral health, including caries. Fluoride is an important element to preventing caries. Relatively little is known about

adults' knowledge, attitudes and behaviors concerning fluoride.

**Objectives:** To investigate knowledge, attitudes, and behaviors concerning fluoride of adults living in Green Hill, Alabama.

**Methods:** A convenience sample of 144 adults aged 18 and older and receiving Green Hill public water at their residence completed online questionnaires through Qualtrics or paper-copy questionnaires (later merged into Qualtrics). Questionnaires assessed knowledge, attitudes, and behaviors concerning fluoride, as well as demographics. The respondents 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 Tau, and one-way ANOVA) to assess associations between age, gender, level of education and knowledge, attitudes, and behaviors.

**Results:** Subjects' mean age was 43.6+/-15.1 years and 26% were male. Ninety-two percent agreed that fluoride prevents cavities, 90% that fluoride does not make people sick, and 87% that fluoride is not harmful to their health. Older age was positively associated with agreement that fluoride should be in tap water (p<0.001) and likelihood of drinking tap water (p=0.018). Lower education was associated with belief

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that fluoride made the water taste funny ( $p=0.003$ ). Respondents' levels of water fluoridation were not significantly associated with any of the variables.

**Conclusion:** The majority of subjects agreed that fluoride was safe and beneficial. Drinking fluoridated water was not associated with other variables. Older adults were more likely to perceive the importance of water fluoridation and drink tap water, while less-

educated adults were more likely to believe that fluoride made the water taste funny. Additional research is needed about the complex relationships among knowledge, attitudes, and behaviors concerning fluoride and relationships with other factors.

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

## Oral Session 5

### 33. Investigating Changes of Referral Patterns to a Pediatric Dental Clinic



**Sara E. Saey**<sup>1</sup>, F. Qian<sup>1</sup>, N.C. Hollinger<sup>1</sup>, M.J. Kanellis<sup>1</sup>, K. Weber-Gasparoni<sup>1</sup>, K. Leary<sup>1</sup>

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

**Objective:** The objective of this study was to compare characteristics of current referred patients with previously referred patients at the University of Iowa Pediatric Dental Clinic (UIPDC). It was hypothesized that there will be a difference in referral patterns between the two groups of patient outcomes. The information obtained from this study will provide a better understanding of the effectiveness of the UIPDC referral system. Understanding if and how referral patterns have changed will allow for better staff coverage, more timely treatment, anticipation of the types of treatment being referred, and will ultimately improve patient care.

**Methods:** Data were previously collected from 340 patients referred to the UIPDC between July 1, 2015 to May 31, 2016. The present study collected data from approximately 1700 patients referred from July 1, 2020 through December 31, 2021 to the UIPDC. Patient information included: age, the duration between the initial appointment and treatment completion, payment source (Medicaid, private insurance, self-pay), driving distance from the patient's hometown to the UIPDC, the size of the patient's hometown, referring provider, and the treatment needs of the patient. Descriptive statistics were generated. Bivariate and multiple regression analyses are forthcoming ( $\alpha=0.05$ ).

**Results:** With the new electronic referral system, a total of 1,611 patients were referred to the UIPDC in the specified treatment period compared to 340 patients in the previous study involving paper referrals. Of the 1,611 referral patients, the mean age was  $6.5\pm 3.1$  years and 73.1% had Medicaid. Nearly 70% of patients were referred by general dentists, and 56.8% lived greater than 60 miles away from the UIPDC. Approximately 90% of patients had caries and the greatest treatment needs were stainless steel crowns (60%), restorations (49%), and primary tooth extractions (46%). The results of comparisons between the two groups of referred patients will be reported when the data processing is complete.

**Conclusion:** Important information was found between referred patient populations. Further data is forthcoming.

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

### 34. Temporomandibular Disorders and Systemic Health in University Students



**An-Vi Phan**<sup>1</sup>, H. Chen<sup>1</sup>, J.J. Warren<sup>1</sup>, W. Shi<sup>1</sup>

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

**Objective:** To explore prevalence of and relationship between TMD-related jaw pain and systemic health conditions in young adults.

**Methods:** An online Qualtrics survey was distributed to current students at the University of Iowa (ages 18-35 years) via email. Self-reported history of jaw pain and systemic health conditions in 3 categories (pain-related issues, sleep issues, and psychological issues) were collected. Descriptive analyses for demographic characteristics and prevalence of jaw pain and systemic health conditions were conducted. P-values were calculated via Chi-square/Fisher's exact tests for categorical variables, and t-test for continuous variables. The associations between TMD-related jaw pain and the presence of systemic health conditions were evaluated by odds ratios. Significance level was at 5%.

**Results:** Of the 27,065 students contacted, less than 10% completed the survey. The majority of survey responders were white (84%), female (81%) and the average age was  $20.8\pm 3.5$  years. Of those who completed the survey, approximately 40% reported jaw pain in the last 30 days, and a majority of students' jaw pain was TMD related. Most also reported having at least one category of systemic health conditions. Those with any systemic health condition were more likely to report jaw pain (odds ratio=1.75, 95% CI= 1.27 – 2.40).

**Conclusion:** TMD-related jaw pain and systemic health conditions are common in university student survey responders. The relationship between TMD and systemic health warrants further research in this population.

Supported by: University of Iowa, College of Dentistry Clinical/Dental Education Research Initiative Support Program. The University of Iowa College of Dentistry Student Research Program.

### 35. Dentist participation in Iowa Medicaid Varied across Managed Care Carriers



**Pamela Nwachukwu**<sup>1</sup>, J.C. Reynolds<sup>1</sup>, P.C. Damiano<sup>1</sup>, S.M. Levy<sup>1</sup>, D. Shane<sup>1</sup>, A. Singhal<sup>2</sup>, S.M. Dabdoub<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA; <sup>2</sup>Boston University, Boston, MA

**Objective:** Dentist Medicaid participation in the literature has been assessed largely as a binary outcome, whereby the dentist either participates in Medicaid or not. Little attention has been paid to the nuances in dentist participation. One factor that has not been studied is the variation in participation across Medicaid dental managed care carriers in states with more than one. This study examines the variation and patterns of dentist participation between the two managed care carriers in Iowa's Dental Medicaid program.

**Methods:** Data were obtained from a survey fielded in August 2021 to Iowa private practice dentists ( $n=1,256$ ). Responding general dentists ( $n=497$ ) were included in the final analytic sample. Descriptive and bivariate analyses were conducted to describe demographic and practice characteristics associated with dentist participation with each of the Medicaid dental carriers. Medicaid participation was defined as dentist-reported acceptance of new adult or child Medicaid patients.

**Results:** Rates of acceptance of new adult Medicaid patients were 26% (carrier 1) and 7% (carrier 2), and for children, they were 40% (carrier 1) and 11% (carrier 2). Medicaid participation was associated with dentists' full or part-time status and practice location, for children. Urban dentists were significantly less likely to accept children with one carrier ( $p<0.001$ ), but not significantly less likely to accept children with the other carrier ( $p=0.88$ ).

**Conclusion:** There was considerable variation in dentist participation between dental managed care carriers in Iowa, as well as across age groups. Most demographic and practice characteristics were not significantly associated with Medicaid participation for either of the carriers for children or adults, except for dentists' work hours and rurality, which was associated with Medicaid participation for children with one carrier. Variation in dentist participation across carriers can considerably limit access to care for Medicaid beneficiaries depending on which carrier they are enrolled in.

Supported by: Iowa Department of Human Services.

### 36. CPP-ACP Paste's Effect on Salivary Conditions in Removable-Denture Patients



**Colton E. Curtis**<sup>1</sup>, R. Bowers<sup>1</sup>, F. Qian<sup>1</sup>

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**Objective:** Denture-wearing older adults experience high rates of both caries (when natural dentition remains) and xerostomia. While many dental therapeutics targeting these two interrelated challenges are commercially available, none have been labeled for use on the intaglio of removable dentures. The peripheral seal of dentures has been hypothesized to provide a unique advantage of retaining therapeutics within the oral cavity for extended time periods. The current clinical trial investigated an off-label use of MI Paste™ (GC America), a dental therapeutic containing casein-phosphopeptide-amorphous-calcium-phosphate (CPP-ACP), under complete dentures and its subsequent effects on salivary buffering capacity and flow rates.

**Methods:** After screening participants for properly fitting complete denture(s), salivary flow rate and pH were recorded at baseline and 15 minutes following the application of CPP-ACP-paste to the intaglio of patients' dentures. To assess buffering capacity, samples were titrated with equivalent volumes of 0.01M lactic acid, and pH reduction was measured. Comparisons of salivary parameters between baseline and post-intervention or between subjects with and without reported xerostomia were conducted using a paired-sample t-test, Wilcoxon signed-rank test, or two-sample t-test as appropriate ( $\alpha=0.05$ ).

**Results:** Of the 28 participants (mean age= $70.3\pm 13.7$  years, 17 males), 11 reported xerostomia. CPP-ACP-paste-intervention was associated with decreased pH reduction during titration compared to baseline ( $0.953\pm 0.241$  vs.  $1.540\pm 0.526$ ,  $p<0.001$ ), and increased pH following titration ( $5.925 \pm 0.343$  vs.  $5.395 \pm 0.660$ ,  $p<0.001$ ). The difference in flow rate between baseline and post-intervention was marginally significant ( $0.547\pm 0.342$  vs.  $0.666\pm 0.443$ ,  $p=0.053$ ). No significant differences in either salivary parameter were found between participants with and without reported xerostomia.

**Conclusion:** The current investigation demonstrates a novel use of CPP-ACP-paste as a potential caries-risk-management tool and dry-mouth aid in a population with complex oral-health challenges. The findings highlight positive effects to salivary conditions. However, determining the caries-preventive clinical significance of the described technique will require longer-term trials; further research is ongoing.

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

### 37. Novel Role For Prmt5 In Oral and Skin Epithelial Development



**Nicole M. Recka**<sup>1</sup>, A. Simons-Burnett<sup>1</sup>, R. Cornell<sup>68</sup>, E. Van Otterloo<sup>1</sup>

<sup>1</sup>University of Iowa, Iowa City, IA;  
<sup>68</sup>University of Washington, Seattle, WA

**Objective:** During development, the initially single-layered epithelium of the skin and oral cavity requires a precise coordination of cell proliferation and differentiation to execute epidermal stratification. Defects in these events can lead to a range of congenital epidermal anomalies. Moreover, the accumulation of harmful mutations can lead to uncontrolled epithelial proliferation, often through reactivation of developmental programs, and carcinoma. Thus, uncovering molecular regulators of epidermal development can provide novel avenues for cancer therapies. 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 cancer 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 driven 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 have used conditional mouse genetics to delete Prmt5 from the early (-E8.5) ectoderm. Additionally, to probe the molecular underpinnings of these defects, we are currently profiling changes in gene expression (RNA-seq), chromatin accessibility (ATAC-seq), and histone methylation.

**Results:** Consistent with a critical role for PRMT5 during this process, epithelial loss of Prmt5 resulted in gross skin and oral epithelial defects, reduced skin barrier function, and reduced postnatal viability. Histological analyses of control and mutant skin revealed severe defects in epidermal stratification, including the loss of the proliferative basal layer.

**Conclusion:** Collectively, our findings have identified a critical role for PRMT5 in epithelial 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: The University of Iowa College of Dentistry P3.

### 38. Prognostic value of PD-L1 Expression in Oral Squamous Cell Carcinoma



**Wattawan Wongpattaraworakul**<sup>1</sup>, A. Choi<sup>1</sup>, M. Buchakjian<sup>1</sup>, E. Lanzel<sup>1</sup>, A. Simons<sup>1</sup>, K.D.A. Rajan<sup>1</sup>

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

**Objective:** Programmed cell death protein ligand-1 (PD-L1) is an immune immunological control leading to tumor cells avoiding immune destruction. In the existing literature, the prognostic value of PD-L1 in OSCC is different among studies. Reasons for contradictory results include different clones of antibody, differences in scoring methods and definitions of positive expression. Here, we aim to evaluate the prognostic role of PD-L1 expression in OSCC using an FDA-approved antibody and the associated recommended scoring criteria.

**Methods:** Tissue microarrays (TMAs) containing 347 OSCC were analyzed for PD-L1 expression by immunohistochemistry (IHC) using antibody clone 22C3 pharmDx. Combined positive score (CPS) criteria were used to evaluate PD-L1 immunoreactivity and correlated with clinicopathologic parameters. The ability of different cutoff points of PDL1 expression to predict progression-free (PFS) and overall patient survival (OS) were evaluated using univariate and multivariate analysis. Associations between immune-expression scores and clinicopathological features were analyzed by Chi-square tests. Survival outcomes were analyzed using Kaplan-Meier plots and log-rank tests.

**Results:** High PD-L1 expression (CPS  $\geq$  10) shows significant associations with absence of active smoking, female sex, and increased patient age. PD-L1 expression did not predict overall survival (OS) or progression free survival (PFS) in patients treated with surgery and radiation. However, in OSCC treated by surgery alone, high PD-L1 expression (CPS  $\geq$  70) was associated with worse PFS. Additionally, high PDL1 expression (CPS  $\geq$  50) was associated with worse PFS in patients with advanced stage (III/IV) OSCC. On multivariate analysis, high PD-L1 expression was associated with worse PFS in OSCC treated by surgery alone with histopathologically confirmed N0 disease.

**Conclusion:** PD-L1 can serve as an independent prognostic marker of PFS in OSCC patients who received surgery alone. This subgroup of patients should be closely monitored, especially those who have advanced stage OSCC.

## Poster Presentations

### 39. Adverse Events Following Use of Nitrous Oxide



**Jeffrey T. Anderson**<sup>1</sup>, K. Leary<sup>1</sup>, P. Iben<sup>1</sup>

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**Objective:** Use of nitrous oxide is common in pediatric dental settings to help improve patient behavior and experience. While nitrous oxide is generally regarded as safe, adverse

events have been reported. The purpose of this study is to identify adverse events that result from the use of nitrous oxide in a dental school.

**Methods:** Data were collected from 9,484 nitrous oxide forms from all departments at the University of Iowa College of Dentistry from August 2, 2017 – July 6, 2022. Any forms missing data were excluded from the study, resulting in 7,554 patient forms analyzed. Information obtained from each form included age, sex, nitrous oxide concentration and duration, department and presence of an adverse event. Patients with adverse events were accessed via the electronic health record for further information.

**Results:** 96.5% of forms reviewed were from the Pediatric Dentistry department. 117 out of 7,554 (0.7%) patients experienced an adverse event across all departments, and 116 (99.1%) occurred in pediatric dentistry. The adverse event group spent an average of 41.8 minutes on nitrous oxide, while the non-adverse event group spent an average of 34.2 minutes on nitrous oxide ( $p < 0.001$ ). Males accounted for 65% of all adverse events. The most common event was “nausea/vomiting”.

**Conclusion:** The results of our study suggest that males have a higher likelihood of experiencing an adverse nitrous oxide event. In addition, more time spent on nitrous oxide appears to increase the odds of an adverse event.

### 40. Fluoride Exposure in Low-Income High-Risk Children



**Ryan Abdelrahim**<sup>1</sup>, A. Lesch<sup>1</sup>, K. Weber-Gasparoni<sup>1</sup>, F. Qian<sup>1</sup>

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

**Objective:** The aim of the study was to assess fluoride exposure from drinking water and toothpaste among low-income high-caries risk children attending the

University of Iowa Infant Oral Health Program (IOHP). An additional purpose was to evaluate the relationship between fluoride source-specific exposures and child's age/race/ethnicity, as well as caregiver's education and socioeconomic status.

**Methods:** Secondary data from the first IOHP visit of 1024 children, 6-36 months of age, consisted of demographic, reported fluoride exposure, oral hygiene practices, and clinical variables were reviewed for a cross-sectional study. Simple and multivariable logistic regression analyses were conducted ( $\alpha=0.05$ ).

**Results:** Ninety-one percent of participating children had never been to a dentist before, 47.4% were female and 69.1% were non-White. It was found that 58% of the children were not exposed to fluoridated water (FW) and 62.8% did not use fluoridated toothpaste (FT). Multiple logistic regression analysis revealed that children not exposed to FW were more likely to be younger (OR=0.98;  $P=.007$ ), Black/African American (OR=2.88;  $P<.001$ ) or be non-Black minority (OR=3.00;  $P<.001$ ), not live with both parents (OR=1.52;  $P=.004$ ) and have caregivers with no previous awareness of ECC (OR=1.71;  $P<.001$ ). Further analysis revealed that those not exposed to FT were also more likely to be younger (OR=0.93;  $P<.001$ ), White (OR=1.82;  $P=.002$ ) or non-Black minority (OR=1.78;  $P=.002$ ), in a single-child household (OR=1.73;  $P<.001$ ), sporadically or never have their teeth brushed (OR=5.80;  $P<.001$ ), and be classified as low-caries risk (OR=2.11;  $P<.001$ ).

**Conclusion:** Education regarding the benefits of daily consumption of fluoridated water and use of fluoridated toothpaste should be targeted to caregivers of low-income young children.

### 41. Gender Differences in Pediatric Dentists' Career Satisfaction: A National Survey



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**Objective:** The purpose of the study was to determine career satisfaction differences between male and female pediatric dentists.

**Methods:** A survey was sent electronically via REDCap to 6,349 active AAPD pediatric dentists. Survey consisted of 29 demographic and practice characteristics and 38 career satisfaction questions based on the 5-point Professional Satisfaction and Dentists' Satisfaction Scales. Bivariate analyses were conducted using a contingency table, chi-square test, and Mann-Whitney U test to detect the difference between male and female pediatric dentists, and a general linear model was used to evaluate the association between overall professional satisfaction and its related factors including personal and professional characteristics, and work environment factors.

**Results:** 674 surveys were returned (11% response rate). When compared to their counterparts, male pediatric dentists were more likely to be older (53.4±13.9 vs. 43.7±10.4 years; p<0.001), Caucasian (88.1% vs. 71.2%; p<0.001), married (91.1% vs. 85.2%; p=0.018), have children (86.2% vs. 73.4%; p<0.001) and a spouse/partner spending more time on common household tasks (50.5% vs. 12.1%; p<0.001), as well as be practice owner/partner (63.4% vs. 50.6%; p=0.002) and treat more patients weekly (126.5±82.7\120.0 vs. 110.2±67.4\100.0; p=0.012). Female pediatric dentists had significantly lower career satisfaction mean ranking scores than males (345.3\3.15±1.37 vs.316.0\2.91±1.45; p=0.024). In addition, they were less satisfied with job/professional satisfaction, income professional time, delivery of care, practice management, staff, respect, and well-being (p<0.05 in each instance). Conversely, male pediatric dentists reported greater satisfaction regarding time for personal life (p<0.001). No significant differences between genders were found regarding community size, primary practice setting, AAPD practice region, patient relations and Covid-19 impact (all p>0.05). The stepwise multiple regression analysis indicated the three most important predictors of overall professional satisfaction were well-being, respect, patient relations after adjusting gender and age.

**Conclusion:** Overall, male pediatric dentists are more satisfied with their career than their female counterparts.

#### 42. Second Molar Substitution: A Survey of Dental Specialists



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**Objective:** To evaluate knowledge, training, and treatment philosophy of dental specialists regarding second

molar substitution, sometimes known as first molar replacement. Respective region and dental specialty were assessed.

**Methods:** A 19-question survey was emailed Spring/Summer of 2022 to active members of the American Academy of Pediatric Dentistry, the American Association of Endodontists, and a sample of the American Association of Orthodontists.

**Results:** 940 complete surveys were received, 58.8% were pediatric dentists, 27.0% endodontists, 13.3% orthodontists, and 1.7% "other". Responders practice in the following area; 21.0% northeastern, 19.8% southeastern, 21.1% north central, 15.6% southwestern, and 22.3% western. Over 75% of responders are in private practice. 87.2% have heard of second molar substitution and 68.0% of those were introduced to the concept in their residency. Of those made aware during residency, 69.3% were pediatric dentists and 15.3% endodontists and orthodontists. Given various

scenarios, 56.2% of responders would only extract the affected tooth rather than extract both the affected tooth and the unaffected opposing or contralateral tooth, but this differed by specialty (P<0.001).

**Conclusion:** Second molar substitution is a well-known concept within dental specialists. Given various scenarios and treatment options, the majority of specialists would treatment plan only the affected tooth or teeth to be extracted rather than opting to extract the opposing or contralateral tooth, if unaffected.

#### 43. 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 Iowa College of Dentistry (UICOD).

**Methods:** A retrospective chart review of health histories and consents was conducted for new patients in the Departments of Pediatric Dentistry and Admission at the UICOD January, 1 through May 31, 2022 that used the PP. Age, gender, size of the community, distance to the UICOD, clinic that the patient is assigned to for treatment, and insurance were obtained. Logistic regression was performed to see if distance from clinic, population of home community, clinic type, insurance type, and whether the patient was referred, can predict whether a patient is more likely to complete their consents and histories through the PP.

**Results:** A total of 121 charts were reviewed: 87 from Admissions and 34 from the Pediatric Clinics. Almost two-thirds of the participants were enrolled in Medicaid, 80% were referred, the average distance traveled was 81 miles and the population of communities that patients came from ranged between 304-214,133. 78% of Admissions patients and 91.2% of Pediatric patients completed all of the consent forms. None of the variables were significant in changing the predicted probability of a patient completing their consents or histories through the PP.

**Conclusion:** This was an exploratory study of characteristics of individuals that completed health histories and consents through the PP. Even though there were no significant characteristics in the regression model, it provides guidance for future research in use of the PP.

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

#### 44. Identification of Biomarker Species Associated with Obesity and Metabolic Syndrome



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**Objective:** With the advent of the Human Microbiome project, the critical role of human microbiome in health and disease is well established. Obesity and Metabolic diseases are emerging non-communicable pandemics affecting more than one-third of the US adult population. Evidence from the gut environment suggests that specific key species are associated with dysbiosis and homeostasis in obesity and Metabolic Syndrome. However, such studies are lacking in the oral environment. Hence, we attempted to identify bacterial indicator species of obesity and Metabolic Syndrome (MetS) in our adult and pediatric patient cohorts.

**Methods:** 208 Saliva and subgingival samples from periodontally, healthy, frequency-matched patients belonging to adult and pediatric obesity and MetS were utilized for this analysis. Bacterial DNA isolated, V3-V4 region amplified, 16S sequencing performed on Illumina Miseq platform, annotated against HOMD database. Indicspecies, DESeq2 packages from R studio, and TukeyHSD were used to determine indicator species and significantly differentially abundant species between the groups. A 2-fold log difference with a p-value less than 0.05 was set as a threshold for significance. Bacterial species that were identified in Indicspecies and that were identified as differentially significantly abundant by DESeq2 and Tukey HSD were determined to be 'indicator species' associated with MetS.

**Results:** We were able to identify potential indicator bacterial species that are associated with adult and pediatric MetS in the oral microbiome. Treponema denticola emerged as an indicator species in pediatric MetS cohort in the salivary environment, while Prevotella scopos was identified as a potential indicator species of MetS in the salivary environment of our adult cohort. Acinetobacter johnsonii was identified as a potential indicator species of Adult MetS in the subgingival environment.

**Conclusion:** Our rigorous statistical analysis demonstrates that pathogenic bacterial species such as Treponema, Prevotella, and Acinetobacter could be identified as keystone species associated with metabolic syndrome, even in the absence of clinical periodontal disease. Our data needs to be further validated in larger cohorts and using mechanistic approaches.

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

#### 45. Oral Hygiene and Dental Care Utilization in Palliative Care Patients



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**Objective:** Increased disease burden, functional disability, and mental distress at the end of life can increase risk of oral disease. Good oral hygiene and regular

dental care are essential to improving oral health and maintaining quality of life. Unfortunately, there is no information regarding the oral hygiene behaviors and dental care patterns in persons receiving palliative care (PRPC) and how these patterns correlate with their oral health status. This study aimed to address this gap.

**Methods:** Forty-nine English-speaking PRPC, aged 18 years or older, were recruited from the University of Iowa Palliative Care Clinic. Participants were interviewed regarding their oral hygiene practices (e.g., toothbrushing frequency, required assistance, and self-rated oral care skills) and dental care patterns (e.g., frequency of dental visits, time, and reasons for last visit). An oral examination was given to assess PRPC's oral health. Analyses were utilized to determine associations between oral hygiene practices, dental care patterns, and oral health outcomes.

**Results:** 49 participants averaged 58.4 years old. 57% were females and 67% had dental insurance. Nearly 60% brushed their teeth 2 or more times per day and their mean plaque score (0.70) and gingival score (0.92) were significantly less than those of the 40.3% who brushed their teeth once a day or less (plaque score=1.09, gingival score=1.17). Participants utilized dental care regularly (46.9% had a dental visit within 6 months and 20.4% had one 6-12 months ago). Nearly one-third (30.6%) last visited a dentist over a year ago. These individuals had significantly more decayed/broken teeth than those with a dental visit within the past 6 months (1.67 vs. 1.19, P=0.03).

**Conclusion:** Despite terminal illness, most PRPC still valued their oral hygiene and utilized dental care regularly, lowering the risk of oral disease.

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

#### 46. Associations between Dietary Caries Risk Assessment Questions and Planned Restorations



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**Objective:** Past research has shown a link between sugar consumption and caries, and sugar intake is a key element in caries risk assessment (CRA). However, our previous research found only weak relationships between sugar intake and completed dental caries treatments. This study's purpose was to assess relationships between dietary questions and planned restorative treatments.

**Methods:** Deidentified data from the University of Iowa electronic dental records (Axium) were used for this study. Specifically, data were obtained on patients who were 18 years or older and completed their initial CRA during 2018-2019. For these patients, specific restorative procedure codes were collected to identify patients who had planned caries treatments 6 months before or after their initial CRA. Whether each patient had any planned caries restorative treatments, and the number of such planned restorations were gathered. Bivariate and logistic regression analyses were conducted to identify factors related to having planned caries treatments.

**Results:** There were 6,218 individuals included in this study, with slightly more females (54%) than males, and the mean age was 51 years. Dietary variables including eating more than 3 snacks per day, having unstructured meals, consuming sugar-sweetened beverages, and drinking sugar-sweetened beverages for more than 30 minutes were found to be significantly related to planned caries treatment ( $p < 0.01$ ). Multivariate models, controlling for age, sex and insurance status, also showed dietary factors to be significantly related to planned caries treatment.

**Conclusion:** In contrast to our previous study of completed treatments, our analysis found dietary factors are strongly related to caries development. Findings suggest that sugar-related questions are valuable in CRA, and that planned treatments may be a better proxy measure for caries in a dental school population.

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

#### 47. Characteristics of Patients Electing Comprehensive versus Single-Visit Care



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**Objective:** In general, new dental patients elect either limited care (LC) or comprehensive care (CC) at their initial visit. To promote the best treatment outcomes, providers often encourage patients to establish CC at that visit. The objective of this analysis was to identify traits and subsequent treatment patterns that differ between those who elect LC versus CC at one university dental clinic.

**Methods:** Data were collected from electronic dental records at the University of Iowa College of Dentistry. The analysis included N=25505 adults who received a screening exam in the Admissions clinic between 1/1/13-12/31/17. Age, gender, preferred language, insurance type, provider type, distance traveled to clinic, and year of screening were assessed at baseline; and follow-up over the subsequent four years yielded the following variables: treatment clinic, time to comprehensive exam, time to first treatment, time to exit exam, first non-diagnostic procedure code, number of visits, and number of procedures.

**Results:** 51% of new patients chose LC at their initial visit, and 34% of LC and 81% of CC patients ultimately received a comprehensive exam. Older age, having private insurance, being enrolled in Medicaid, speaking English or Spanish, living further from the clinic, and being screened more recently were associated with electing CC over LC ( $p < 0.001$ ). Most often, first non-diagnostic procedures post-screening were preventive, periodontic, prosthodontic or orthodontic for CC patients, versus endodontic or surgical for LC patients.

**Conclusion:** These findings identify groups that could be targeted to increase the election of comprehensive care at the initial visit.

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

#### 48. Evaluation of Implant Position: A Comparison of Two Imaging Modalities



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**Objective:** Dental implants are an increasingly popular choice for replacement of missing teeth due to many factors including increased longevity, improved aesthetics, and maintenance of existing tooth structures. In order to maintain peri-implant health, ensure longevity of the implant, and protect other structures in the oral cavity such as nerves and sinuses, the endosteal implant must have accurate surgical placement. There is no standard of care to indicate imaging required before implant surgery. This retrospective study aims to establish a standard of care by comparing the accuracy of implants with a pantomograph (pano) taken before surgery compared to those implants placed with cone-beam computed tomography (CBCT) imaging taken before surgery.

**Methods:** All cases in the study had CBCT scans taken at the University of Iowa College of Dentistry after implants were placed, and either a CBCT (Group A) or pano (Group B) taken before implants were placed. A single examiner assessed the accuracy of implant placement in Group A compared to implant placement in Group B by analyzing the CBCT of the implants after surgery. After exclusion criteria were considered, Group A contained 82 cases (212 individual implants) and Group B contained 76 cases (165 individual implants). Each individual implant was categorized as "Within Normal Limits (WNL)" or "Not Within Normal Limits (Not WNL)".

**Results:** Implant site specific analytics show Group A had more implants placed WNL than Group B in posterior teeth, particularly in mandibular posterior teeth where 82% of mandibular molars in Group A were WNL compared to only 67% in Group B and mandibular premolars in Group A were 88% WNL compared to 79% WNL in Group B.

**Conclusion:** This study concludes that for mandibular posterior teeth, a CBCT image prior to endosteal implant placement ensures greater accuracy and WNL placement.

#### 49. Patient Payment Method Influence on Orthodontic Treatment Completion



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**Objective:** To understand how patient payment method influences completion of orthodontic treatment.

**Methods:** This study includes all orthodontic patients from July of 2009 to June of 2019 that began treatment at the University of Iowa in the Orthodontic Residency Clinic. Patients were divided into three sub-groups based on their primary method of payment for orthodontic services: private insurance, state-funded insurance, or self-pay. We excluded patients with a complicated medical history, cleft lip/palate or syndromes, cases that required orthognathic surgery or especially complex orthodontic treatment needs such as severe eruption delays.

**Results:** 1,624 patients were included in the study: 1,138 had private insurance, 236 had state-funded insurance, and 250 patients were self-pay. No association was found between payment method and orthodontic treatment completion. Treatment completion time for patients with state-funded insurance and patients with private insurance was significant ( $p=0.0471$ ). Failed appointments between both state-funded insurance and private insurance ( $p=0.0001$ ) and state-funded insurance and self-pay methods ( $p=0.0002$ ) is also significant. No significance was found between payment method and orthodontic treatment completion rates, distance in miles from home to clinic, appointment cancellations, consent deband of orthodontic treatment, or biological sex.

**Conclusion:** Payment method does not influence completion rates of orthodontic treatment. Instead, treatment completion time is the most influential variable on whether a patient will complete orthodontic treatment. Patients with state-funded insurance are more likely to fail appointments compared to patients with self-pay or patients with private-insurance. However, failed appointments do not influence completion rate of orthodontic treatment.

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## 50. Private Practice Dentist Comments on Transition to DWP Kids



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**Objective:** Iowa's Medicaid dental program for children transitioned from state fee-for-service to privatized managed care in 2021 and was named the Dental Wellness Plan (DWP) Kids. The objective of this study was to assess common themes among dentist survey comments related to this transition.

**Methods:** The data source for this study was a survey sent to all private practice dentists in Iowa in August 2021 to assess dentist experiences and attitudes regarding Iowa's Medicaid dental program. This study used data from two open-ended items from this survey related to the transition to DWP Kids: 1) "Do you have any comments about the transition to DWP Kids?" and 2) "If your acceptance of new child patients changed as a result of the transition to DWP Kids, what are the main reasons why it changed?" Responses were qualitatively analyzed and coded to determine common themes.

**Results:** 46% (n=564) of dentists participated in the survey. Of those, 25% responded to the first question and 14% responded to the second. For the first question, the most common themes were reimbursement, administrative burden, carrier issues, and the inability to restrict Medicaid participation to children only. For the second, the most common themes were reimbursement/cost and contract language, including concerns about child acceptance being tied to adult acceptance.

**Conclusions:** In a qualitative analysis of comments from a survey sent to private practice dentists, the most common themes related to the transition to Medicaid managed care for kids were reimbursement, administrative burden, carrier issues, and contract issues.

Supported by: Iowa Department of Health and Human Services.

## 51. ADHD Knowledge and Stigma Evaluation in the College of Dentistry



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**Objective:** ADHD is a very prevalent neurodevelopmental disorder in the United States. Among medical students, ADHD accounts for the greatest proportion of disclosed disabilities, yet remains considerably lower than the expected prevalence. This gap in disclosure and support-seeking may be due to stigma surrounding psychological disorders, especially

about adult ADHD in higher education. Few studies exist in dental school populations; thus, this study aims to assess general knowledge and stigma about ADHD in the College of Dentistry.

**Methods:** A Qualtrics survey was created and distributed to all COD members via email (n=1,318). The anonymous survey was available for one month and consisted of 9 T/F knowledge items and 19 stigma items about Consequences of Diagnostic Disclosure, Malingering and Misuse of Medication, and Ability to Take Responsibility. Selections were based upon reported experiences and stressors for students with ADHD. Statistical analysis consisted of descriptive, univariate, and multivariate regression analyses (p<0.05).

**Results:** 269 participants completed the survey with a 20.4% response rate. Knowledge and stigmatizing attitudes did not statistically differ by age or role (student, resident, faculty, staff). Of the 9 knowledge items, participants yielded a mean of 6.9 correct items, but had important misconceptions about diagnosis and symptoms. Only 52.08% correctly answered "ADHD is a disorder present from childhood" and 40.9% incorrectly answered "People with ADHD find it difficult to follow rules." Total knowledge was negatively correlated with total stigma. Participants who did not know anyone with ADHD responded with statistically higher levels of total stigma of medium effect size (d=0.57). Further multivariate analyses are forthcoming to evaluate hypothesized cumulative stigma decrease with increasing personal connections.

**Conclusion:** Although ADHD knowledge was high overall in this sample, misconceptions and stigmatizing attitudes about ADHD persist. Having a personal connection to someone with ADHD was the most important factor correlating to reduced stigma.

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

## 52. Assessing Behavior Guidance Modalities of Pediatric Patients With Teledentistry Consultations



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**Objective:** The purpose of the study was to determine the change in behavior guidance modality (BGM) for pediatric dental patients from an initial teledentistry consultation through treatment completion and identify associations with clinical and demographic factors. BGMs include treatment in clinic with or without nitrous oxide, oral conscious sedation, or general anesthesia.

**Methods:** 79 children (43 male, 36 female) and their caregivers completed synchronous teledentistry consultations with pediatric dental residents between

July 1, 2020 and June 4, 2021. The electronic dental records (axiUm) of referral patients to the University of Iowa College of Dentistry for specialty care were reviewed to collect data on recommended BGM, BGM used to complete treatment, and clinical and patient related factors. Descriptive and bivariate analyses were conducted (alpha=0.05).

**Results:** Initial behavioral modality for treatment did not change for 86% of children. Dental treatment planned for in the clinic had the most changes in BGM followed by sedation. Patients planned for general anesthesia had no change in BGM. A change in BGM from teledentistry consultation to treatment was always to a more advanced modality. More complex treatment plans were associated with having a change in BGM (24% 4 or more quadrants vs 6.7% 3 or fewer quadrants; p=0.048).

**Conclusion:** Teledentistry correctly identified the BGM for most pediatric dental patients. Treatment complexity was associated with a change in BGM. This study may help dental practitioners understand the limitations of teledentistry when accessing BGM for a patient with 4 or more quadrants of dental care.

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

## 53. Effects of Pre-Doctoral Experiences on Treating Patients with Alzheimer's Disease



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**Objective:** Patients with Alzheimer's Disease (AD) face many barriers that prevent access to dental care including finding dentists who are willing and able to provide care as their AD progresses. The aim of this study was to investigate the effect of pre-doctoral educational experiences on Alumni's attitudes and willingness to provide care for patients with AD.

**Methods:** An electronic survey was distributed to all University of Iowa Dental Alumni in August 2022. Respondents included both alumni who did and did not experience a 4-5 week pre-doctoral clinic rotation that provided comprehensive care to patients with AD. The survey topics include provider demographics, pre-doctoral clinical AD experiences, practitioner's current comfort level with AD post-graduation, and a validated Dementia Attitude Scale (DAS). Wilcoxon rank-sum, chi-squared, and Fisher's exact tests were used for analysis.

**Results:** Of the 75 respondents who completed the survey, 49 (65.33%) provided care in the Geriatric and Special Needs (GSN) clinic and/or the Geriatric Mobile Unit (GMU), 22 (29.33%) stated they had no clinical experience. A majority of those stated that they either agreed or strongly agreed that GSN and GMU

experience positively contributed to preparedness to treat patients with AD (95.65% and 92.50% respectively). 95.92%, 85.71%, and 75.51% of those who received pre-doctoral education stated that they would be comfortable providing care to patients with early-stage, middle-stage, and late-stage AD respectively. As a limitation to analysis, 84.72% of providers are treating 5 or fewer patients with AD per month in their current setting.

**Conclusion:** Bivariate analysis showed no statistical significance between predoctoral clinical experience and no clinical experience on current attitudes and willingness to provide care for patients with AD due to the limited number of respondents. However, most felt that predoctoral education experiences positively contributed to their ability to provide care for these patients.

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

## 54. Long-Term Oral Health Outcomes for Removable Partial Denture Patients



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**Objective:** To explore the long-term oral health outcomes of patients receiving removable partial dentures and identify patterns of tooth loss, decay, and additional prosthodontic treatment.

**Methods:** Adults (n=7399) received removable partial dentures from the COD between 2009 and 2018. Electronic health records (EHRs) of these patients were reviewed and treatment completed after delivery of the RPD was recorded using current dental terminology (CDT) codes to indicate categorized treatments. Demographic data were also collected.

**Results:** The EHR identified 3746 (51.3%) female, 3601 (48.7% male) and 2 (0.0%) unspecified individuals in the cohort. 24% of patients were enrolled in the Dental Wellness Plan, 43% were identified as self-pay, and 34% had private dental insurance. Additional treatments averaged, in CDT codes per patient: 15.27 preventative, 8.40 direct restorative, 1.425 fixed prosthodontic, 0.47 endodontic, 8.24 surgical, 3.46 removable prosthodontics. Overall, patients averaged 37.26 additional treatments.

**Conclusion:** A large population of patients who received removable partial dentures was described. These patients require preventive care and can have further dental treatment needs such as crowns, extractions, and root canal treatment for remaining teeth. This may have implications for future treatment of partially-edentulous patients.

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

## 55. Modifications on Dentin Mechanical and Biochemical Properties Promoted by Silanes



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**Objective:** To characterize potential interactions between silanes and dentinal collagen.

**Methods:** Dentin blocks (3 x 3 x 0.5 mm) and slabs (0.8 mm-thick) were prepared from coronal dentin of human extracted molars. Slabs were trimmed in two orientations, perpendicular (PP) or parallel (PL) to the tubules, to produce hour-glass shaped specimens with 0.8 mm at the mid-coronal dentin. Specimens were demineralized in 0.5 M EDTA for 3 days at 4°C followed by treatment with PBS (control), 100 mM 3-(Triethoxysilyl)propyl isocyanate (ICPTES), 100 mM (3-aminopropyl)triethoxysilane (APTES), or 5% glutaraldehyde (GD) for 1 h. Changes in dentin mechanical properties were assessed by ultimate tensile strength (UTS) test using the slabs (n=7), and results were expressed in megapascal (MPa). Fourier-transform infrared spectroscopy (FTIR) with attenuated total reflectance (ATR) was used to characterize biochemical modifications in the dentin blocks (n=3). Intensity of assigned peaks was obtained from the FTIR spectra before and after treatment. After normalization, ratios between amide I/CH<sub>2</sub> (1,640 cm<sup>-1</sup>/1,450 cm<sup>-1</sup>), amide II/CH<sub>2</sub> (1,550 cm<sup>-1</sup>/1,450 cm<sup>-1</sup>), and amide III/CH<sub>2</sub> (1,240 cm<sup>-1</sup>/1,450 cm<sup>-1</sup>) were calculated. The Kruskal-Wallis test with Bonferroni correction, Mann-Whitney, and one-way ANOVA tests were performed for statistical analyses (α=0.05).

**Results:** Statistically significant differences were observed between PBS-treated dentin tested PL and APTES-treated samples tested in PP orientation (p=0.006). When comparing UTS tested PP and PL to the tubules, ICPTES and GD treatment resulted in no statistically significant differences (p=0.406 and p=0.565). Treatment with ICPTES, APTES, or GD resulted in decreased amide I/CH<sub>2</sub> and amide II/CH<sub>2</sub> in comparison to the control; however, no statistically significant differences were observed among the different treatment for any of the calculated ratios (p>0.05)

**Conclusion:** Similar to GD, ICPTES promoted changes in dentin's UTS and anisotropy, which might suggest potential interactions with collagen. Additional characterization of specific interactions between collagen and silanes is required.

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

## 56. Outcomes of Silver Diamine Fluoride (SDF) Treatment Among Older Adults



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**Objective:** In this project, we aim to analyze the results of SDF outcomes using a retrospective cohort design. This project's results may improve clinicians' ability to predict the success of SDF treatment in adults 65 or older.

**Methods:** We collected data from patients at the University of Iowa College of Dentistry who were 65 years or older with SDF application on a tooth (n=1083) from 2016 to 2018 with a minimum three-year follow-up. The data was accessed from the electronic health record, Axium. From these 1083 observations of SDF application, there are 865 unique combinations of patients/sites (some patients had multiple treatments on the same site), and 437 unique patients.

**Results:** 66.8% of patients (n = 292 patients) had no further codes of interest on the same site after the day of their first SDF procedure in the specified time frame, while 33.2% (n = 145 patients) did. Of the 33.2% who did return for further treatment, the most common procedures were D1110 (prophylaxis), D7140, and D2391.1 (glass ionomer type restorations). Other less-frequent codes (each representing fewer than 1% of total procedures) represented 24% combined. About 44% of known codes were preventive and 56% were non-preventive; however, many codes were not categorized so this may not be the full picture. Glass ionomer-type restorations and examinations were the most common types of procedures.

**Conclusion:** In this population of adults who are 65 years old and older, the majority of patients had no further treatment at the College of Dentistry after the first SDF procedure in the specified time frame of 2016 to 2018.

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

## 57. Bond Strength of an Adhesive System Containing Proanthocyanidin-Loaded Nanoparticles



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**Objective:** To assess the immediate and long-term bonding performance of an experimental resin-based primer (RBP) containing mesoporous silica nanoparticles (MSN) loaded with proanthocyanidins (PACs).

**Methods:** The mid-coronal dentin of extracted intact human molars was restored using different RBP (n=10): Control (no MSN), MSN-APTES (RBP containing MSN functionalized with 3-aminopropyltriethoxysilane (APTES), MSN-APTES-PAC (RBP containing MSN loaded with PAC after functionalization), and MSN-PAC-APTES (RBP containing MSN loaded with PAC before functionalization). After the RBP application, a commercial adhesive resin was used (Scotchbond Multipurpose, 3M Oral Care) followed by a resin composite (Filtek Supreme Ultra, 3M Oral Care). Four resin-dentin beams were obtained per tooth and two specimens were immediately tested in tensile until failure. The remaining two specimens were tested after 1 year of storage. Failure mode was classified as adhesive, cohesive, or mixed. Statistical analysis consisted of mixed modeling for repeated measures with a Tukey post-hoc and Weibull distribution with a Sidak pairwise test for the bond strength data. Failure mode was analyzed by Fisher exact tests. All analyzes were performed using R (v. 4.1.2) (α=0.05).

**Results:** For immediate results, MSN-PAC-APTES presented statistically significant higher bond strength than the other groups (all pairwise p<0.03). No statistically significant difference was observed among the other three primer formulations (p=0.93). Comparing the long-term to the immediate results, Control and MSN-PAC-APTES showed a decrease in bond strength after 1 year of storage (p=0.027 and p=0.002, respectively). However, MSN-APTES-PAC resulted in a significant increase in bond strength after the long-term storage (p<0.001).

**Conclusion:** APTES-functionalized MSN can be successfully added to a primer for drug-delivery purposes without compromising the bond strength to dentin. However, the sequence of surface functionalization with APTES resulted in differences in the bonding performance, with better long-term results for RBP containing MSN loaded with PAC after functionalization.

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## 58. Characteristics of Admissions Patients at the UI College of Dentistry



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**Objective:** This study describes selected demographic and health conditions among patients attending their initial visit at the UI College of Dentistry in the Admissions Clinic, with specific interest in patients who requested comprehensive care (CC) versus urgent care (UC).

**Methods:** Demographic information and health conditions of de-identified patients seen during the years 2016-2021 were analyzed. Patients aged 18 or older were included in the analysis. Univariate distributions were assessed, and range checks were performed, followed by bivariate analyses comparing characteristics of those whose posted ADA treatment codes were D0140.4 (CC screening exam) versus D0140.1 (UC screening exam).

**Results:** Data from 31,876 patients were analyzed. Patients' average age was 43 years and 53% were women. Self-reported categories of race were "White/European" (85%), "Black/African" (10%), and "Other" (4.7%). From 2016 to 2021, the percent of privately insured patients decreased from 30 to 22%; self-paying patients decreased from 39% to 25%; and Medicaid / DWP patients increased from 31% to 52% (p<0.001). More patients in the CC group reported diabetes (20% vs 16%), joint replacement (8.4% vs 5.6%), heart disease (37% vs 29%), and muscular/bone concerns (17% vs 13%) compared to UC patients (all p-values <0.001). More patients in the CC group reported having seen a primary care provider in the year prior to the appointment compared to the UC group (77% vs 64%, p <0.001).

**Conclusion:** Patients who requested comprehensive care reported more adverse health conditions (diabetes, joint replacement, heart disease, muscular/bone concerns) and were more likely to have seen a primary care provider in the previous year than did those who requested urgent care. Differences between the two groups should be investigated further in longitudinal studies, as they might have implications for teaching and patient care in the College of Dentistry's Admissions Clinic.

### 59. Student Empathy With Standardized Patients: Concepts From Critical Thinking Emulation



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**Objective:** Clinician empathy can improve patient outcomes, but the literature is scant on patient-based,

student-led experiences to demonstrate empathy in patient interactions. The purposes of this project are to (1) develop a learning guide for observable behaviors communicating emotional and cognitive empathy, and (2) determine whether the learning guide can be used as a rubric for assessing empathy in a standardized patient experience.

**Methods:** Eleven standardized patients assessed 80 D3 students using a 4-point interval scale on 19 behavioral criteria in four domains: Initiation (four criteria); Health History and Caries Risk (four criteria); Treatment Planning (six criteria); and Communication Skills (five criteria). Standardized patients also provided qualitative feedback.

**Results:** Standardized Patients completed all 1,520 interval scales on the rubric and 94% of 320 open ended entries. Students performed well. Of the 1,520 criterion interval scales, 1,242 (81.7%) were rated "excellent." Students performed best in Treatment Planning, with 411 (85.6%) of 480 criteria rated "excellent." Health History had the most room for improvement, with 248 (77.5%) of 320 criteria rated "excellent." In keeping with the scores, qualitative feedback was overwhelmingly positive for Treatment Planning and more equivocal for Health History.

**Conclusions:** The emulation model for students to demonstrate observable aspects of empathy is viable as both a learning guide and evaluation rubric in a Standardized Patient format. Next steps include continuation of the standardized patient format, development of a succinct skillset for reinforcement in the patient setting and continued discussion on what best captures core observable aspects of empathy.

### 60. Prevalence of Fluoride Varnish Application in the Medical Setting



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B. Levy<sup>1</sup>, M.A. Keels<sup>121</sup>, A. Hara<sup>12</sup>,  
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Dental caries is prevalent in children and fluoride varnish (FV) applications are an effective preventive strategy. FV in medical settings offers an opportunity for caries prevention, but few studies have assessed its use in medical settings.

**Objective:** To assess the cross-sectional and 18-month cumulative prevalence of FV application in medical settings using a convenience sample of subjects from 3 sites.

**Methods:** After IRB approval, 1,326 primary caregiver/child pairs were enrolled at child age 12 months in 2012-2013 for a study developing a caries risk assessment tool. Children received dental examinations and parents completed self-administered questionnaires at baseline, and at 30, 48, 78, 96, and 114 months of age that asked about medical FV application. Every four months, intermediate questionnaires asked about medical FV application. Cumulative prevalence for four 18-month periods were calculated, and percentages of all responses from age 12-30 months with medical FV were calculated.

**Results:** The majority of children aged 2.5 years were male (51.1%), white (50.8%), non-Hispanic (87.2%), and Medicaid-insured (54.1%). Cumulative prevalence of 1+ FV applications was 20.4% for age 12-30 months, 12.9% for 30-48 months, 6.4% for 78-96 months, and 1.9% for 96 to 114 months. The distribution of the percentage of responses with medical FV application from 12-30 months was: 79.6% had no FV (0%), 6.4% had 20-33% of responses with medical FV, 9.1% had 40-67% with medical FV, and 4.9% had 75-100% of responses with medical FV.

**Conclusion:** Prevalence of FV application in the medical setting is low and decreases as children age.

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### 61. Fracture Resistance Bonded Zirconia Crowns After Endodontic Access Hole Preparation



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**Objective:** Approximately 15% of natural teeth receiving full zirconia crown coverage present pulp vitality changes and root canal complications. Endodontic access holes produce residual stress during access preparation due to poor heat conduction of zirconia and cracking susceptibility. Alterations during endodontic access opening in all-ceramic crowns have shown edge chipping around access openings and radial crack formation. This study evaluates Null Hypothesis (HO): There are no fracture resistance changes on bonded or luted zirconia crowns with varying occlusal thicknesses after endodontic access hole preparation.

**Methods:** 120 extracted molars were prepared, scanned and designed in 3D Shape CAD software with different occlusal thicknesses of 1.0 mm, 1.5 mm and 2.0 mm. Crowns were wet milled using zirconia containing 3 mol% yttria (3Y Ceramill) then cemented using Resin Modified Glass Ionomer (Relyx) and Resin-based (Panavia). Thermo-cycling test (5,000 cycles, 5°C-55°C) confirmed lifecycle aging. Endodontic access was prepared under microscope. Resin composite restored access holes (Filtek Supreme Ultra, 3M ESPE). All samples were tested in Universal testing machine. Fracture load was measured at the maximum load until catastrophic fracture; calculated in Newtons (N).

**Results:** Larger occlusal thicknesses require more fracture force. A 1 mm increase in occlusal thickness increased average zirconia crown fracture force by 1043.00 N. Endodontic access hole decreases average crown fracture force by 653.83 N. Force needed to fracture zirconia crowns using Panavia cement is expected to be 1600.75 N greater than for a crown using Relyx. Rejected Null hypotheses. T-tests against null hypotheses of no effect indicate significant main effects of the thickness and access hole. Increased occlusal thickness increases fracture force. Access hole presence decreased fracture force.

**Conclusion:** Selection of occlusal thickness influences the strength of zirconia crowns. Increase in occlusal thickness can add crown strength. After endodontic treatment, zirconia crowns yield clinically relevant fractures and failures.

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### 62. A Multifunctional Hydrogel Enhances Osteogenesis Via TLR4/NF-kB/HO-1 Axis



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**Objective:** Effective therapies capable of inhibiting inflammation and promoting bone healing remain to be developed for inflammatory bone diseases. Global transcriptomic changes demonstrated inflammatory responses dominate periodontitis with bone loss. While anti-inflammation treatment can enhance bone formation at defects, development agent can synergistically repair periodontal tissues. Consequently, this study aimed to engineer a multi-functional hydrogel to restore the abnormal inflammation environment and promote bone regeneration for periodontal tissue regeneration.

**Methods:** We fabricated anti-inflammation and anti-oxidation nanomicelles (PP5 NMs) by self-assembly method. Then, we evaluated their ROS-scavenging and anti-inflammatory effects, and explore their anti-oxidation, anti-apoptosis, anti-inflammation and pro-osteogenesis capacity on human periodontal ligament cells (hPDLs). Next, we engineered a multi-functional hydrogel via co-loaded with PP5 NMs and a pro-osteogenic agent recombinant human BMP9 (rhBMP9) based on a thermo-sensitive polymer PX and observed its physicochemical characterization. Then, we establish rats with mandibular and cranial defects model to observe bone formation capacity of the hydrogel. Finally, transcriptome sequencing was applied to further explore the mechanism of PP5 NMs reserving inflammation on hPDLs.

**Results:** We successfully engineered PP5 NMs and validated their ROS-scavenging and anti-inflammatory effects. Meanwhile, we demonstrated that PP5 NMs promoted hPDLs' osteogenesis by anti-oxidation, anti-apoptosis, and anti-inflammation. Then, our results validate this engineered multifunctional hydrogel successfully utilizes the synergistic effects of PP5 NMs and rhBMP9 and itself could effectively facilitate bone formation at mandibular and cranial defects, by attenuating local inflammation/oxidative stress and enhancing local stem cell osteogenic differentiation potential. Mechanistically, we verified the inflammation/oxidation-resolving capacities of the hydrogel are mainly achieved by PP5 NMs via TLR4/NF-κ B/HO-1 axis to improve self-regeneration of hPDLs.

**Conclusion:** We rationally engineered an injectable temperature-sensitive hydrogel, which possesses temperature-triggered gelling performance, anti-inflammation and anti-oxidation characteristic (PP5 NMs), and osteogenesis-promoting capability (rhBMP9). Such treatment strategies can be further applied as an injectable delivery vehicle for different types of inflammatory bone disease.

### 63. Chemerin/ChemR23 Promotes Inflammatory Root Resorption in Mice via Cementoblast Senescence



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**Objective:** Inflammatory root resorption (IRR) is a common phenomenon in patients undergoing orthodontic treatment, with an unclear pathogenesis.

Increased inflammatory mediators are associated with cellular senescence. Chemerin induces senescence in human nucleus pulposus cells (NPC) and releases inflammatory mediators that may play a role in IRR. We aimed to explore possible roles of the chemerin/ChemR23 interaction in cementoblast senescence and IRR and reveal a new IRR therapeutic target.

**Methods:** Detection of cementoblast senescence phenotypes including P53, P21, P16 and pro-inflammatory senescence-associated secretory phenotypes (SASP) by qRT-PCR and Western blotting after chemerin and siChemR23 action on the immortalized murine cementoblast cell line OCCM-30. Detection of senescent cementoblast by immunofluorescence with  $\beta$ -galactosidase and EDU. Observation of changes in acetylated histones in cementoblast in the presence of Chemerin using the sirt-1 specific agonist resveratrol. In addition, we established IRR models in 2-, 8-, 12-, and 16-month-old C57BL/6J mice and injected mice with chemerin and ChemR23 antibodies. Then, the amount of root resorption was calculated, the expression of P53, P21, IL-1 $\beta$  and IL-6 in periodontal tissues was detected, and the serum chemerin levels in mice of different ages were measured.

**Results:** Chemerin induces senescence and acts as a pro-inflammatory agent on cementoblast, and these effects can be partially reversed by siChemR23. Chemerin treatment of cementoblast resulted in decreased Sirt1 expression and increased expression of h3k9ac, h3k27ac, P53, P16, and P21, effects that were partially reversed by resveratrol. It is suggested that sirt-1 is a downstream molecule of chemerin/ChemR23 and affects histone acetylation and thus cellular senescence. *In vivo*, there were age-increasing changes in serum Chemerin concentrations, and IRR worsened after Chemerin injection. expression of P53, P16 was positively correlated with Chemerin. Downregulation of ChemR23 partially reversed these effects.

**Conclusion:** Chemerin/ChemR23 induced cementoblast senescence and thus affected root resorption by inhibiting sirt1, highlighting the therapeutic potential of chemerin in IRR.

### 64. Cancer Cell Membranes Functionalized Nanoparticles for Targeted Oral Cancer therapy



**Hongjiao Li**<sup>101</sup>, D. Yang<sup>101</sup>

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**Objective:** Oral squamous cell carcinoma (OSCC) is a prevalent malignant disease worldwide due to the lack of an efficient strategy for targeted therapy. To solve

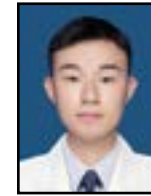
this problem, a combined chemo/photothermal therapy nanoplatfrom was constructed for enhanced OSCC therapy.

**Methods:** Based on cancer cell homotypic targeting ability, biomimetic nanoparticles (CC@DOXNPs) were fabricated with HSC3 membrane (CC) coating on the surface of DOX-loaded poly (lactic-co-glycolic acid) copolymer (PLGA) nanoparticles (DOXNPs). Then, indocyanine green (ICG) modified with the phospholipid polymer DSPE-PEG2000 was inserted into the cancer cell membrane for photothermal therapy. Subsequently, the physical properties of the various nanoparticles were characterized. The biocompatibility and biological functions of nanoparticles were determined *in vitro*. Finally, in cancer subcutaneous and orthotopic models, the targeting characteristics, therapeutic efficacy and safety of the nanoparticles were examined.

**Results:** CC-coated nanoparticles effectively targeted and accumulated tumor areas *in vivo*. The lip-CC@DOXNPs achieved synergistic chemotherapy and photothermal in cancer cells upon laser irradiation. After 2-week treatment program, lip-CC@DOXNPs were shown to significantly inhibit the progression of tumor. Furthermore, our nanoparticles displayed favorable safety performance after long-term administration.

**Conclusion:** Taking together, our combined nanoplatfroms have tumor targeting and drugs deliver capacities, as well as the photothermal conversion ability, making them an ideal for synergistic chemo/photothermal therapy against OSCC. This proof of concept may provide a smart therapeutic system in the field of targeted chemo/photothermal therapy therapeutic platforms.

### 65. Copper-containing Bioglass-Hydrogel With Hemostatic, Antibacterial, Angiogenesis Properties for Wound Healing



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**Objective:** Full-thickness skin injury, followed by massive bleeding and wound

infection, is a difficult clinical problem. Therefore, biomaterials are urgently needed to treat severe skin defects to achieve rapid hemostasis and extensive tissue remodeling. The purpose of this study is to construct a copper-containing bioglass (Cu-BGn) loaded gelatin methacrylate (GelMA) composite hydrogel (GelMA@Cu-BGn) to promote the healing of full-thickness skin defects.

**Methods:** Rat liver injury and tail amputation models were established to test the hemostatic effect of the material. The cell viability of GelMA@Cu-BGn hydrogel were evaluated using mouse fibroblasts (L929). And the scratch assay and tube formation experiment were carried out by using L929 and human umbilical vein endothelial cells (HUVEC) to evaluate the angiogenesis ability of GelMA@Cu-BGn hydrogel. In addition, *Staphylococcus aureus* and *Escherichia coli* were used to test the antibacterial effect of GelMA@Cu-BGn hydrogel. The effect of GelMA@Cu-BGn hydrogel on wound healing was evaluated *in vivo* using a diabetic rat full-thickness skin defect model.

**Results:** Compared to commercial collagen sponge, GelMA@Cu-BGn hydrogel exhibited shorter hemostatic time and lower blood loss in both rat liver injury and tail amputation models. Meanwhile, GelMA@Cu-BGn hydrogel with 1% concentration of Cu-BGn showed good biocompatibility and outstanding angiogenesis property. Furthermore, GelMA@Cu-BGn hydrogel exhibited nontrivial antibacterial effect towards both *Staphylococcus aureus* and *Escherichia coli*. Most importantly, GelMA@Cu-BGn hydrogel exhibited better healing effect than the commercial dressing Tegaderm3M in full-thickness skin defects of diabetic rats.

**Conclusion:** Taken together, the results of this experiment verify that GelMA@Cu-BGn hydrogel has excellent hemostatic effect, biocompatibility, antibacterial and angiogenesis properties, and is a potential promising wound dressing for diabetic wound healing.

### 66. Gut Dysbiosis Caused by Antibiotics Aggravates Periodontitis



**Xulei Yuan**<sup>101</sup>, F. Zhou<sup>101</sup>, H. Wang<sup>101</sup>, X. Xu<sup>101</sup>, S. Xu<sup>101</sup>, C. Zhang<sup>101</sup>, Y. Zhang<sup>101</sup>, M. Liu<sup>101</sup>, Y. Zhang<sup>101</sup>, M. Zhou<sup>101</sup>, H. Li<sup>101</sup>, X. Zhang<sup>101</sup>, T. Zhang<sup>101</sup>, J. Song<sup>101</sup>

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**Objective:** This study aimed to investigate whether gut dysbiosis caused by long-term systemic antibiotic use affects periodontitis.

**Methods:** Four-week-old male C57 mice were randomly divided into two groups: N and Abs groups. Mice in the Abs group were provided with antibiotic drinking water containing cefoxitin, gentamicin, metronidazole, and vancomycin. After four weeks, the antibiotic drinking water was removed and mice were divided into four groups: N+N, N+Lig, Abs+N, and Abs+Lig groups. An experimental periodontitis model was established in the N+Lig and Abs+Lig groups for two weeks. Other mice were provided with antibiotic drinking water for four weeks. Then, the antibiotic water was removed and mice were divided into two groups: FMT-N and FMT-Abs. The fecal microbiota of mice in the N+N and Abs+N groups were transferred to the FMT-N and FMT-Abs groups, respectively. Two weeks after the transplantation, all mice received a ligature for another two weeks.

**Results:** In gut and oral microbiota, community composition was significantly changed and histological destruction was observed in gut tissue. Compared with N+Lig group, in Abs+Lig group, alveolar bone loss was increased. Fecal microbiota transplantation from normal mice partially restored the disturbed gut microbiota in mice with antibiotics using, and improved intestinal histological destruction. Compared with the FMT-Abs+Lig group, the alveolar bone loss was decreased in the FMT-N+Lig group.

**Conclusion:** Long-term systemic antibiotics-induced gut dysbiosis has potential adverse effects on periodontitis. The periodontal condition of patients should be assessed regularly when using systemic antibiotics in clinical practice.



## 67. Network Between Mitochondrial Dysfunction and Immune Microenvironment of Periodontitis



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**Objective:** To reveal the cross-talk between mitochondrial dysfunction and the immune microenvironment of periodontitis

**Methods:** Public data were acquired from MitoCarta 3.0, Mitomap, and NCBI GEO databases. Hub markers were screened out by five integrated machine learning algorithms (Univariate logistic regression, LASSO, RF, SVM-RFE and XGBoost) and verified by laboratory experiments. Single-cell sequencing data were utilized to unravel cell-type specific expression levels of hub genes. An artificial neural network model was constructed to discriminate periodontitis from healthy controls. An unsupervised consensus clustering algorithm was conducted to reveal mitochondrial dysfunction related periodontitis subtypes. The immune cell, immune related pathways, Mitopathways scores of each sample were calculated using CIBERSORTx and ssGSEA algorithms. The correlation coefficients between hub genes and characteristics of immune microenvironment of periodontitis were evaluated as well.

**Results:** Differentially expressed mitochondria-related genes were identified, and two hub markers (CYP24A1 and HINT3) were screened out by five integrated machine learning algorithms. Single-cell sequencing data revealed that HINT3 was mainly expressed in dendritic cells, while CYP24A1 was mainly expressed in monocytes. An artificial neural network model based on hub genes was constructed and validated, exhibiting robust diagnostic performance. Two distinct mitochondrial phenotypes were unraveled by an unsupervised consensus clustering algorithm, and significantly different pathway activities were discovered between them. These hub genes were found to be associated with the degree of immune cell infiltration in periodontitis subtypes, mitochondrial respiratory chain complexes, and mitochondrial damage-associated molecular patterns.

**Conclusion:** In brief, two hub markers were identified, which may serve as new potential immunotherapy targets, and the concomitant findings of the study could provide a novel reference for future mitochondria-related research of periodontitis.

## 68. Research of the Association of *Candida albicans* With Alzheimer's Disease



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**Objective:** Alzheimer's disease (AD) is a common neurodegenerative disease. The etiology of AD is unknown, and some studies suggest that its pathogenesis is related to microbial imbalance. *Candida albicans* can invade the brain parenchyma through bloodborne infections, causing A $\beta$  deposition, which ultimately leads to learning and memory decline in mice. Extracellular vesicles (EVs) are a series of membrane-bound vesicles secreted by eukaryotic cells. *Candida albicans* is able to secrete extracellular vesicles, and studies have shown that its outer membrane vesicles are associated with biphasic transformation and virulence regulation. We speculate that extracellular vesicles may be an important cause of AD caused by *Candida albicans*. Our study aimed to reveal whether *Candida albicans* and its EVs can lead to pathological changes in Alzheimer's disease and to investigate the mechanism.

**Methods:** We inoculated 14-month-old mice orally with *Candida albicans* and its EV, and after two months, the mice were subjected to behavioral testing, and their hippocampal tissues were collected for relevant molecular biological analysis to detect pathological changes related to Alzheimer's disease from gene and protein expression levels.

**Results:** Our study shows that *Candida albicans* and its EVs can lead to decreased learning and cognitive decline in mice, causing hippocampal neuroinflammation in mice, and the presence of Tau protein increased phosphorylation at the Thr231.

**Conclusion:** *Candida albicans* is a common fungus in the mouth, which can induce neuroinflammation leading to the occurrence of Alzheimer's disease, suggesting the importance of antifungal therapy in the treatment of Alzheimer's disease, and the elderly with denture stomatitis should be more prevented. *Candida albicans* EVs has been reported to be immunogenic, and its specific mechanism for inducing Alzheimer's disease is unclear, there is still research promise as a vaccine to prevent related diseases.

## 69. Responsive Nanoparticle Ameliorates Periodontal Destruction Via Restoring Oxidative Stress Resilience



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**Objective:** Periodontitis is a widespread oral disease characterized by continuous inflammation of the periodontal tissue

and irreversible alveolar bone loss. The periodontal microenvironment is altered by inflammation, which in turn affects cell fate, function, and metabolism by regulating the activity of oxidative stress.

**Objective:** In order to restore the oxidative stress resilience (OxSR) programs and enhance buffering against oxidative damage, we developed an innovated diagnostic and therapeutic nanoparticle loaded with itaconate (ITA), a mitochondrial metabolite, to effectively inhibit the inflammatory process and remodel the periodontal soft and hard tissue by regulating the homeostasis of periodontal microenvironment and reprogramming of macrophages metabolism.

**Methods:** In mice modeling, we applied the silk sutures with both ends tied knots to insert between the maxillary first molar and second molar. The ligature was retained for 2 weeks in order to establish experimental periodontitis. Radiographic analysis and immunostaining were performed post-operation. Molecular docking simulation and knockout mice were used to verify the molecular mechanism of ITA. Then, nanoparticle-based drug delivery has been administrated to improve pharmacokinetics of ITA and to reduce the side effects induced by local high concentrations.

**Results:** We showed that ITA could significantly alleviate inflammation and alveolar bone loss *in vitro* and *in vivo*. Molecular docking simulation revealed a potential mechanism between ITA and Nrf2 activation. Additionally, this was further confirmed in Nrf2 - / - mice, where ITA treatment failed to protect against periodontitis-induced alveolar bone dysfunction due to the absence of Nrf2. The responsive nanoparticle which loaded ITA further demonstrated its therapeutic effect on periodontitis.

**Conclusion:** Our results indicated that the diagnostic and therapeutic nanoparticle loaded with ITA attenuates inflammation and oxidative stress via disassociation of KEAP1-Nrf2 and activation of Nrf2 signaling cascade. Local administration of nanoparticle provides clinical treatment potential for periodontitis.

## 70. Tooth Germ Repair Is Controlled by mTORC1 Signaling



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**Objective:** Tooth germ injury can lead to tooth dysplasia, cysts and even tooth loss. Therefore, understanding the cellular and molecular mechanisms of tooth germ

repair and regeneration is of great significance for the treatment of oral diseases.

**Methods:** Zebrafish tooth germ was specifically injured using NTR/Mtz system. The repair process of tooth germ was observed by TUNEL staining, antibody staining, in situ hybridization staining, RT-PCR, EdU staining, alizarin red staining and time-lapse photography. Finally, the conservation of mTORC1 signaling was verified in mouse.

**Results:** Mtz treatment induced apoptosis in dlx2b+ cells. Mtz treatment for 48 h could specifically ablated zebrafish tooth germ, and the fluorescence almost completely disappeared. Interestingly, the fluorescence of the tooth germ reappeared after removal of Mtz. At the early stage after Mtz removal, the expression of mTOR was significantly increased as screened by RT-PCR. Moreover, the phosphorylation of 4E-BP1, a major protein in mTORC1 signaling pathway, was also increased. These results indicated that mTORC1 signaling was activated at the early stage of tooth germ repair. And we used rapamycin, a specific inhibitor of mTORC1, to inhibit mTORC1 could inhibited tooth germ repair and cell proliferation. Genetic knockout of mTORC1 also observed that the damaged tooth germ did not repair and cell proliferation was significantly reduced after Mtz treatment. In the mouse model of incisor repair after mechanical damage, rapamycin could inhibit incisor repair. Leucine, a promoter of the mTORC1 pathway, could promote the repair of damaged incisor in mouse.

**Conclusion:** We constructed a stable and repeatable model of the repair of tooth germ after severe injury in zebrafish. Using this model, we found that mTORC1 signaling may promote the effective tooth germ repair by regulating cell proliferation. In addition, we demonstrated that mTORC1 signaling also plays a crucial role in regulating the repair of injured incisor in mouse.

## 71. Comparison of Biofilm Models for Producing Artificial White Spot Lesions



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**Objective:** Establishing an effective protocol for creating artificial white spot lesions (WSL) that mimic *in vivo* lesion characteristics opens many possibilities for laboratory studies to investigate novel minimally invasive strategies to arrest lesion progression. This *in vitro* study compared three protocols for developing artificial active WSL using biofilm models associated with enamel demineralization and remineralization cycles.

**Methods:** Forty-five enamel samples were sterilized and divided into three groups based on the biofilm-induced challenge: *Streptococcus sobrinus* with *Lactobacillus casei* (Ss+Lc) for 4 days, *Streptococcus sobrinus* (Ss) for 16 days, or *Streptococcus mutans* (Sm) for 12 days. Bacterial species were grown on the surface of saliva pellicle-coated enamel specimens, and the specific demineralization/remineralization protocols were performed with daily media changes. The fluorescence, depth, and chemical composition of the WSLs were assessed using Quantitative Light-induced Fluorescence (QLF), Polarized Light Microscopy (PLM), and Raman Spectroscopy, respectively. Statistical analysis consisted of two-way ANOVA followed by Tukey's post hoc tests ( $\alpha=0.05$ ).

**Results:** WSLs created using the Ss+Lc protocol presented statistically significant higher fluorescence loss ( $\Delta F$ ) and integrated fluorescence ( $\Delta Q$ ) in comparison to the other two protocols ( $p < 0.05$ ). In addition, Ss+Lc resulted in significantly deeper lesions (137.5  $\mu\text{m}$ ), followed by Ss (84.1  $\mu\text{m}$ ) and Sm (54.9  $\mu\text{m}$ ) ( $p < 0.05$ ). While high mineral content was observed in sound enamel, WSLs created with the Ss+Lc group showed the highest demineralization and exposure of organic content among the three protocols.

**Conclusion:** All the microbial models were suitable for developing active WSLs. However, the microbial model using *S. sobrinus* and *L. casei* for four days resulted in active WSLs with great depth, lower fluorescence, and quicker and more significant demineralization when compared to the other protocols. This protocol also preserves the surface layer and obtains a subsurface lesion that mimics the characteristics of natural lesions.

Supported by: Brazilian Federal Foundation for Support and Evaluation of Graduate Education (CAPES).

## 72. Microhardness and Micropermeability Evaluation of (Glass-Ionomer) Restorative Materials



**Madeleine Reed**<sup>1</sup>, E. Teixeira<sup>1</sup>, A. Leme-Kraus<sup>1</sup>

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

**Objective:** To evaluate the effectiveness of restorative materials in acidic environments the material's micropermeability and hardness of the surrounding dentin and enamel were measured.

**Methods:** Sound teeth were collected, and two cavity preparations were completed on the lingual and buccal side of each tooth in the cervical region. Each cavity preparation was filled with one of the corresponding materials: DeltaFil, Equia Forte HT, Ketac Molar Quick, Surefil one, and Scotchbond Universal Plus + Filtek Bulk Fill. Each tooth was then cut into two, nail polish was applied leaving 2 mm around the restored area exposed. For every tooth, one half would undergo a 7-day pH cycling procedure (6 hours demineralization solution and 18 hours remineralization solution) while the other half was placed in the remineralization solution for the same time at 37°C. Each half was then cut, embedded in epoxy resin, and polished using SiC abrasive papers #320, 600, 800, and 1200. The specimens were stained for 1 hour using 0.1 M Rhodamine-B with a pH of 7.2 and observed under a confocal laser scanning microscope. Micropermeability was determined using a He-Ne laser (514 nm wavelength captured) which determined the amount of rhodamine present in the margins of the restored site of the enamel and root dentin. Microhardness was observed on the enamel and root dentin around the restored site.

**Results:** Using statistical analysis, a significance between the enamel and root dentin was found ( $p = 0.024$ ). It was determined that significantly more micropermeability existed in the restoration using Ketac Molar than Surefil One ( $p = 0.008$ ). No significance was found between the other materials ( $p > 0.05$ ). There was no difference among restorative materials for microhardness ( $p > 0.05$ ). A significant difference was observed between the root dentin and the enamel for microhardness ( $p = 0.001$ ).

**Conclusion:** The hybrid restorative material had the best marginal sealing.

## 73. Differences in Acid-Tolerant Bacteria and *C. albicans* in Children



**Ryan Hemsley**<sup>1</sup>, M. Zhu<sup>1</sup>, A. Villhauer<sup>1</sup>, J.A. Banas<sup>1</sup>

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

**Objective:** Dental caries is known to be a multifactorial disease. Recent studies have suggested a role for acid tolerant bacterial strains, as well as a role for *Candida albicans* in the development of early childhood caries (ECC). Statistical differences are typically evident when comparing caries and caries-free populations. But caries risk assessment is best carried out at the level of the individual. The purpose of this study was to compare the recoveries of: 1) mutans streptococci (MS), 2) *C. albicans*, and 3) acid-tolerant strains between toddlers with caries and age and gender matched caries-free toddlers, and to evaluate the data both collectively and individually. These microbial measures have previously been found to become evident/elevated 12 to 18 months before a caries diagnosis.

**Methods:** Twenty four subjects under 48 months of age were recruited to participate in this study. Dental plaque samples were collected with a sterile cotton-tipped applicator over the coronal surfaces. Plaque samples were cultured on Anaerobic Blood Agar for determination of the total cultivable count, on SB20 agar for the quantification of MS, on CHROMagar Candida for the quantification of *Candida* species, and on Acid Agar (pH 5.0) for the quantification of acid tolerant strains.

**Results:** Collectively, children with caries had higher proportions of *C. albicans*, and statistically higher levels of MS and acid-tolerant strains than did children without caries. At the individual level, all 12 toddlers with caries had detectable *Candida*, MS at  $\geq 0.01\%$  of the total count, and/or an acid tolerant recovery of  $\geq 1\%$  of the total count, whereas only 5 of 12 caries-free subjects met at least one of these criteria.

**Conclusion:** Current caries risk assessment is better at predicting future health than future caries. A focus on multiple microbiological measures may improve the sensitivity of caries risk assessment.

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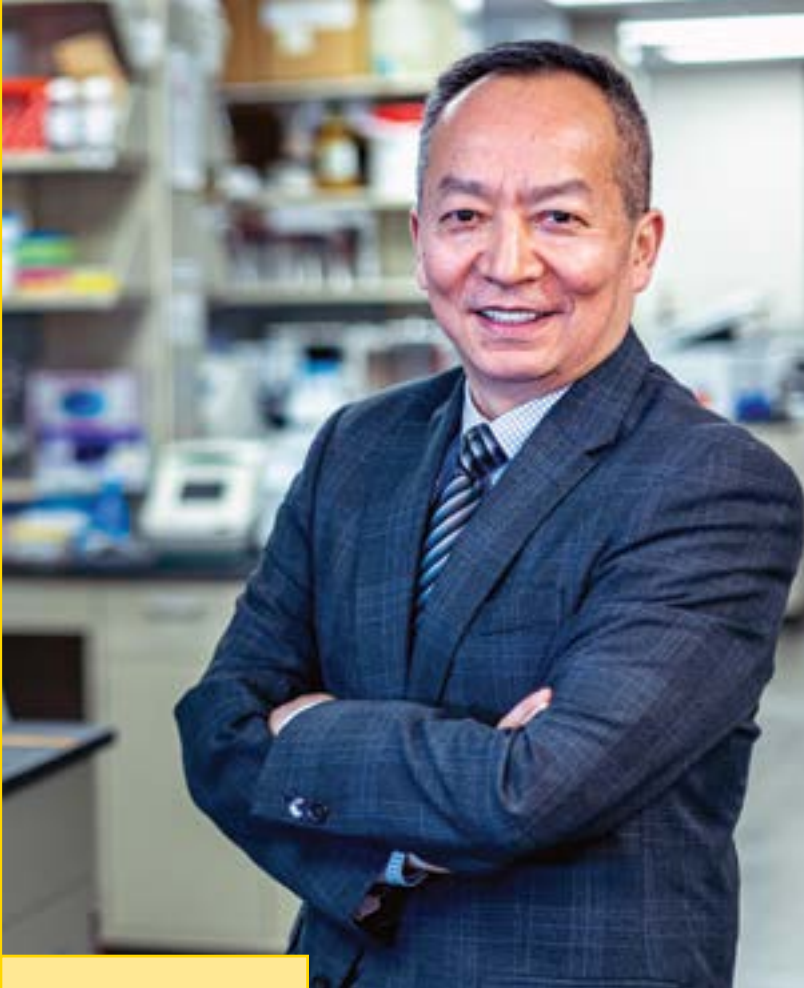
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## Oral Cancer Program ...continued from page 4

The holistic approach of the team encompasses the entire spectrum of oral cavity cancer research, including microenvironmental and genetic predispositions and causes of oral cavity cancer, and its prevention, detection, diagnosis, prognosis, treatment, and recovery. This approach always started with the end point of improving patient outcomes in mind.

In February 2021, the University of Iowa awarded the OCP a \$600,000 grant to further develop the infrastructure, develop preliminary research data as part of the program, and prepare to apply for a Specialized Program of Research Excellence Grant from the National Institutes of Health.

"I am so proud of what our team has accomplished already, but the future of the oral cancer program is even brighter as Iowa is poised to be an international and national leader in oral cavity cancer research due to the team's extraordinary work over the last four years," Xie exclaimed with pride.



*Xian Jin Xie, associate dean for research, is accomplishing his goal of making Iowa a global leader in oral cavity cancer research.*

## Teixeira ...continued from page 5

"These companies not only provide valuable funds and equipment to support research, but they also align with the research team interests and ultimate goals."

Universities provide a strong statement in industry-university partnerships. An independent expert in the field conducting their analysis using a new technology ensures that a business's profit-motive is autonomous of the research findings. This process provides good scientific evidence and helps establish public trust in the quality of a new technology element.

However, this process depends primarily on the integrity and expertise of the liberated researcher. SONDENDO approached Dr. Teixeira and his team precisely for these reasons.

"It is paramount that researchers maintain their autonomy from external sponsors," Teixeira said,

"since it protects both the integrity of the researcher and the reputation of the company."

Unfortunately, this process began just as the COVID-19 pandemic hit early 2020. As a result, the research project experienced several delays and is still developing a sufficiently large patient pool to complete the study.

Teixeira's team includes statisticians Xian Jin Xie and Carissa Comnick, Sara Miller as clinic coordinator, and endodontics residents and faculty using the technology at the endodontic clinic.

Teixeira said, "I am fortunate to have worked with such wonderful people on this project, notably Sara Miller, Ann Lawler, Sharon Seydel and Carissa Comnick. We are very enthusiastic about this translational research opportunity."

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