RESEARCH DAY 2022

OFFICE OF RESEARCH
FEBRUARY 25, 2022

POSTER PRESENTATIONS:
go.osu.edu/cdorresearchday2022

ZOOM REGISTRATION:
dentistry.osu.edu/research/research-day

THE OHIO STATE UNIVERSITY
COLLEGE OF DENTISTRY
# SCHEDULE OF EVENTS

## SPEAKERS PROGRAM

### Opening Remarks: John D. Bartlett, PhD
Associate Dean for Research, College of Dentistry

8:15-8:25 a.m.

### Faculty Speaker: Carroll Ann Trotman, BDS, MA, MS
Dean, College of Dentistry
Professor, Division of Orthodontics
Title: “The Power of the Face: Restoring Paralysis and Clefting”

8:25-8:55 a.m.

### Distinguished Lecturer Introduction: John D. Bartlett, PhD
Associate Dean for Research, College of Dentistry

8:55-9:00 a.m.

### Distinguished Lecturer:
Sarah M. Knox, PhD, Associate Professor in the Department of Cell & Tissue Biology at the University of California, San Francisco, School of Dentistry.

9:00-10:00 a.m.

“Regenerating Epithelial Organs Using the Autonomic Nerve Supply”

1.5 CDE credits (all speakers) available for licensed practitioners

## AWARDS PRESENTATION

### Dean’s Awards for Excellence in Research
Scott R. Schricker, PhD, Associate Professor, Director of Student Research
John D. Bartlett, PhD, Associate Dean for Research, Professor

10:00-11:00 a.m.
ORGANIZING COMMITTEE

Dean
Carrol Ann Trotman, BDS, MA, MS

Associate Dean for Research
John Bartlett, PhD

Director, Student Research Programs,
Advisor to Student Research Group
Scott Schricker, PhD

College Research Committee Chairperson
Binnaz Leblebicioglu DDS, MS, PhD

Research Day Planning & Support
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James Moulton, Web Communications Specialist
Tammarra Steedman, Director

OHIO STATE DENTISTRY
STUDENT RESEARCH GROUP

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Kazune Pax (DDS/PhD year 5)

Vice President
Michelle Scott (DDS/PhD year 5)

Secretary
Natalie Andras (DDS/PhD year 3)

Treasurer
Mark Boyce (D3)

Advocacy Rep
Stella Kim (D4)

Historian
Aakriti Chaudhry (D2)
As Dean of the College of Dentistry, I am pleased to welcome you to our annual Research Day. This event provides an opportunity for our students, faculty, and guests to learn about the scientific achievements and innovations from our college. It also presents exciting discoveries made by some of our world-renowned researchers in dentistry and oral health. This year like last year, Research Day is a virtual event designed to maintain the safety of all our participants. If safety permits, we plan to have our traditional in-person events in the future.

This year’s distinguished lecturer is Sarah M. Knox, PhD. She is the Assistant Dean for Research and Associate Professor in the Department of Cell & Tissue Biology at the University of California, San Francisco, School of Dentistry. Her research was recently recognized by receipt of the 2021 IADR Distinguished Scientist Award for Salivary Research, and by being awarded an NIH funded sustained outstanding achievement in research (SOAR) grant.

Dr. Knox’s research focuses on the mechanisms through which glandular organs develop, age and regenerate after injury, how autoimmune disease affects regenerative processes, and the impact of the nervous system on cancer. We are pleased and honored to welcome her as our distinguished lecturer, and we look forward to her presentation on “Regenerating epithelial organs using the autonomic nerve supply.”

Our Research Day includes presentations that showcase the research conducted by our dental students, post-doctoral fellows, and residents. This part of the event offers a comprehensive view of the diverse research activities in which the members of our college are engaged. To recognize those whose research has been judged as outstanding, we will present these individuals awards at a virtual celebration via Zoom.

Please join me in thanking Dr. John Bartlett, Associate Dean for Research, and the Office of Research staff members who have contributed their collective time and efforts in preparing this exceptional program that provides us all with an enriching and worthwhile experience.

Carroll Ann Trotman, B.D.S., M.A., M.S.
Dean and Professor
WELCOME

FROM THE ASSOCIATE
DEAN FOR RESEARCH

As the Associate Dean for Research, it is my pleasure to welcome you to the College of Dentistry’s 38th annual Research Day! This event celebrates our students’, staff’s, and faculty members’ commitment to research pursuits, and it also provides an opportunity for us to learn about each other’s research interests.

Many of our faculty have graciously taken the time to judge student presentations for the Research Day Awards Ceremony; Dean’s Awards will be presented for the best research projects for the top DDS students, top graduate students and residents, and for the top hygiene student and postdoctoral fellow.

Research Day prepares our students and postdoctoral fellows for national and international competitions, including several sponsored by the American or International Association for Dental Research. Top presenters qualify to compete at the AADOCR/Dentsply Student Clinical Program and the nationally recognized Hinman Student Dental Research Symposium.

Additionally, awards are generously presented by the Dentsply Sirona and the American Association for Dental Oral and Craniofacial Research. Moreover, faculty researchers are honored by the College of Dentistry with Paper of the Year Awards for Basic Research, Clinical Research, and Social and Behavioral Sciences & Public Health Research.

This year, Carroll Ann Trotman, BDS, MA, MS, is our Research Day faculty presenter. She is a Professor in the Division of Orthodontics and is the Dean of The Ohio State University College of Dentistry. Dean Trotman studies facial mobility and recovery after surgery on patients with facial paralysis and with cleft lip and palate. Since 2001, Dean Trotman has been a Principle Investigator or Co-Principle Investigator on eleven different grants funded by the National Institutes of Health (NIH) and has served on a number of NIH study sections to review grant applications. Dean Trotman has been awarded several honors for her research pursuits including the ADA NDAF Colgate Faculty Recognition Award for Research and she was elected to the Robert R. Andrews Honor Society for the Promotion of Research at Tufts University. Her talk is titled, “The Power of the Face: Restoring Function Following Paralysis and Clefting”.

We are pleased to welcome Sarah Knox, PhD, as our Distinguished Lecturer. Dr. Knox earned her doctoral degree in Biomedical Engineering from the University of New South Wales, Sydney, Australia. She is currently the Assistant Dean for Research and is an Associate Professor in the Department of Cell & Tissue Biology at the University of California, San Francisco, School of Dentistry. Dr. Knox studies the mechanisms through which glandular organs develop, age and regenerate after injury; how autoimmune disease affect regenerative processes and the impact of the nervous system on cancer. Dr. Knox has received a variety of awards, the most recent of which is the 2021 IADR Distinguished Scientist Award for Salivary Research. She has had continuous NIH funding since 2012 and was recently awarded a prestigious SOAR grant by the NIDCR. This grant is awarded to researchers with sustained outstanding achievement in research (SOAR). We are thrilled to have Dr. Knox as our Distinguished Lecturer for our 38th Research Day. Her presentation is titled, “Regenerating Epithelial Organs Using the Autonomic Nerve Supply”.

Please join me in extending a sincere thank you to the faculty, staff, and students whose efforts have made this Research Day possible. Good luck to the participants, and my sincere thanks to all of you for joining this event.

JOHN D. BARTLETT, PhD
On behalf of the Ohio State University College of Dentistry Research Committee, I welcome you to Research Day 2022.

Research Day at our college has been a traditional annual event celebrating our students’ research activities since late 1980s. Every year we come together to listen our guest lecturers and our young researchers who are at different stages of their careers. Throughout the dental education curriculum, we heavily emphasize evidence-based practice, and it is always inspiring to learn that some of this knowledge is originating from our own faculty and students. We share the excitement and enthusiasm of clinical and bench type research conducted within our college by the help of dedicated mentors and supportive administration. This year is not an exception; we are having a virtual research day for the second time around due to pandemic related challenges, but this does not diminish our enthusiasm.

Our distinguished speaker Dr. Sarah Monica Knox is joining us from the University of California, San Francisco. She has significant published work on craniofacial development and salivary glands. She is covering the topic of “Regenerative epithelial organs using the autonomic nerve supply”. We also have a chance to listen Dean Trotman and learn about her research for the first time. Of course, our students are spending significant time to put their short presentations together and get ready for this event. We cannot wait to share all these with all of you.

We are hoping that by this time next year, we can go back to an in-person meeting, which allows us to have lots of discussion opportunities between presentations. Until then, please join me to support our young researchers and to enjoy a day full of new data coming out of our college’s laboratories and clinics.

BINNAZ LEBLEBICIÖGLU DDS, MS, PHD
WELCOME

FROM THE RESEARCH COMMITTEE CHAIR

On behalf of Student Research Group (SRG), I am excited to welcome you to the annual Research Day! SRG collaborates with The Ohio State University College of Dentistry every year to provide an opportunity for dental and dental hygiene students, graduate students, postdoctoral fellows, and residents to showcase their research. Although we were hoping to be in person this year, we are excited to put on another virtual Research Day nonetheless! We incorporated feedback from last year so hopefully this virtual Research Day is received even more positively. Our faculty, staff, and students have continued to work hard this year and we want to take this day to recognize their work and perseverance. In addition to learning about the research being conducted by the College, we have the incredible opportunity to learn from amazing scientists who have graciously donated their time to share their noteworthy discoveries with us.

This year, Sarah Knox, PhD, will discuss the regeneration of epithelial organs. Dr. Knox is an associate professor in the School of Dentistry at the University of California, San Francisco. Her lab focuses on epithelial organogenesis and regeneration following injury. She is extremely accomplished and comes highly recommended by her collaborators. I have also had the privilege of meeting Dr. Knox in-person and can attest to her ability to give an engaging talk; she has so much energy and makes you want to listen. She is a genuinely fun person to chat with so if you ever cross paths with her in the future, be sure to say hello!

We also have the honor of learning from our own Dean Carroll Ann Trotman, BDS, MA, MS. Dean Trotman is a professor in the Division Orthodontics. Her research interests revolve around the treatment of patients with cleft lips and palates as well as patients with facial paralysis. We are excited to learn more about a different side of our new Dean!

Furthermore, the presentations given today help prepare the students for national research competitions at the American Association for Dental and Craniofacial Research/International Association for Dental Research (AADOCR/IADR), the ADA/Dentsply Student Clinician Program, and the Hinman Student Dental Research Symposium. Students receive valuable feedback from both senior researchers and distinguished faculty. We applaud all the participants for their tremendous effort to complete their research and present their work. Students recognized for their outstanding achievements will be awarded following the speaker program.

Finally, please join me in extending our sincerest gratitude to Dr. John Bartlett, Associate Dean for Research, the Office of Research staff, and SRG members who help make this event possible. We began planning this as an in-person event and switched to virtual halfway through. Everyone dedicated a lot of time and effort, especially with the swap, to prepare an extraordinary program to provide us all with a meaningful and inspiring experience.

KAZUNE PAX, DDS/PHD CANDIDATE
Carroll Ann E. Trotman BDS, MA, MS, is a Professor in the Division of Orthodontics and is the Dean of The Ohio State University College of Dentistry. Dean Trotman earned her dental degree from the University of Dundee Dental School, Dundee, Scotland and subsequently earned two Master’s degrees. The first was in Oral Biology from Columbia University, Graduate School of Arts and Sciences and the second was in Clinical Research Design and Statistical Analysis at the University of Michigan, Horace Rackham School of Graduate Studies.

Dean Trotman studies facial mobility and recovery after surgery on patients with facial paralysis and with cleft lip and palate. She has had numerous national and international speaking invitations including at the Chinese Orthodontic Conference, the AEEDC Dubai World Orthodontic Conference, the Asian Pacific Orthodontic Conference, the Gordon Conference on Craniofacial Morphogenesis and Tissue Regeneration held in Barga, Italy, and the American Academy of Dental Science at Harvard University. Dean Trotman has served as the Assistant Dean for Graduate Education & Academic Development at the University of North Carolina, the Associate Dean for Academic Affairs and Student Affairs at the University of Maryland, and the Associate Dean for Faculty Development and Chair of the Department of Orthodontics at Tufts University. Since 2001, Dean Trotman has been a Principle Investigator or Co-Principal Investigator on eleven different grants funded by the National Institutes of Health (NIH) and has served on a number of NIH study sections to review grant applications. Dean Trotman has been awarded several honors for her research pursuits including the ADA NDAF Colgate Faculty Recognition Award for Research and she was elected to the Robert R. Andrews Honor Society for the Promotion of Research at Tufts University.

We are excited to learn about Dean Trotman’s facial mobility research during her Research Day presentation titled, “The Power of the Face: Restoring Function Following Paralysis and Clefting”."
Sarah M. Knox, PhD, is an Associate Professor in the Department of Cell & Tissue Biology at the University of California, San Francisco, School of Dentistry. She is currently the Assistant Dean for Research and is the Vice Chair of the Dentistry School’s Faculty Council. Dr. Knox earned both her bachelor’s degree in Biochemistry and her doctoral degree in Biomedical Engineering from the University of New South Wales, Sydney, Australia. Dr. Knox studies the mechanisms through which glandular organs develop, age and regenerate after injury, how autoimmune disease effects regenerative processes and the impact of the nervous system on cancer. She has had numerous national and international speaking invitations including presenting as the keynote speaker at the 10th European Symposium on Saliva, Egmond aan Zee, The Netherlands and was selected as the plenary speaker for the 44th Annual Society for Craniofacial Genetics and Developmental Biology. Dr. Knox has served as the President of the IADR Salivary Research Group, Co-Chair of the Gordon Research Conference on Salivary Glands and Exocrine Biology, and has served on several National Institute of Health (NIH) study sections for grant application reviews. She is a standing member of NIDCR Oral Dental Craniofacial Sciences (ODCS) study section. She has two patent applications and she has published in prestigious journals such as, Development, Developmental Cell, eLife, EMBO Molecular Medicine, FEBB Letters, Nature Communications, and Science, Dr. Knox has received a variety of awards, the most recent of which, is the 2021 IADR Distinguished Scientist Award for Salivary Research. She has had continuous NIH funding since 2012 and was recently awarded a prestigious R35 grant funded by the NIDCR. The R35 grant provides long-term support for mid-career researchers with sustained outstanding achievement in research (SOAR). It is therefore a distinct honor to be awarded a SOAR grant and we are thrilled to have her as our Distinguished Lecturer for our 38th Research Day. The title of her talk is, “Regenerating Epithelial Organs Using the Autonomic Nerve Supply”. 
Our sincere appreciation to the following individuals:

### Student Research Awards Committee

- Dr. Scott Schricker, Chair
- Dr. Kim Hammersmith
- Dr. Homa Amini
- Dr. Yuan-Lynn Hsieh
- Dr. Elisandra Reyes-Perez
- Dr. Beau Meyer
- Dr. Justin Kasper
- Dr. Anjum Shah
- Dr. Kazune Pax
- Mr. Don Gray

### Research Committee

- Dr. Binnaz Leblebicioglu, Chair
- Dr. Justin Kasper
- Dr. Peter Reiser
- Dr. Sarah Peters
- Dr. DoGyoon Kim
- Dr. Melissa Drum
- Dr. Shareef Dabdoub
- Dr. Ehsan Azadani
- Dr. Hany Emam
- Dr. Luiz Meirelles
- Dr. Kazune Pax
- Dr. John Bartlett

And to our distinguished judges for graciously volunteering their time and expertise for this event:

- Dr. Rafat Amer
- Dr. Homa Amini
- Dr. Nidhi Arora
- Dr. Ehsan Azadani
- Dr. Cliff Beall
- Dr. Stephen Beestra
- Dr. Robert Busto
- Ms. Beth Chartier
- Dr. Daniel Claman
- Dr. Lewis Claman
- Dr. Bryant Cornelius
- Dr. Toru Deguchi
- Dr. Melissa Drum
- Dr. Hany Emam
- Dr. Brian Foster
- Dr. Sara Fowler
- Dr. Diogo Gouveia
- Dr. Erin Gross
- Ms. Rebecca Henderson
- Dr. Justin Kasparr
- Ms. Rachel Kearney
- Dr. Do-Gyoon Kim
- Ms. Denise Kissell
- Dr. Binnaz Leblebicioglu
- Dr. Damian Lee
- Dr. Luiz Meirelles
- Dr. Debra Mendel
- Dr. Matthew Messina
- Dr. Beau Meyer
- Dr. Sarah Mikhail
- Dr. Sarah Peters
- Dr. Peter Reiser
- Dr. Scott Schricker
- Dr. Shilpa Shah
- Dr. Zongyang Sun
- Dr. Dimitris Tatakis
- Dr. Susan Travers
- Ms. Bridget Wright

Special thanks to William Johnston, PhD for statistical assistance
The College of Dentistry’s 37th Annual Research Day, held on February 23, 2021, featured dental and dental hygiene students, pre-doctoral students, postdoctoral fellows, and residents who presented abstracts on an array of cutting-edge research topics. The event included poster displays where researchers presented their findings for faculty members, guests, and judges.

Included in the awards for the 2021 Student Research Competition was the SCADA Award co-sponsored by Dentsply Sirona and the American Association for Dental Research (AADR). The winner of this award qualified to be entered into the national Student Competition for Advancing Dental Research and its Application at the annual AADR General Session. The 2021 SCADA Award winner was Kazune Pax.

The second-place award for the Student Research Competition is the Alumni Research Merit Award. The winner of this 2021 award qualified to be entered into the Hinman Student Research Symposium competition, with support from the Thomas P. Hinman Dental Society, the National Institute of Dental and Craniofacial Research, and Procter & Gamble. The 2021 Alumni Research Merit Award winner was Michelle Scott.

The third-place award for student researchers is the Alumni Research Achievement Award. The winner is qualified to enter into the Hinman Student Research Symposium competition. The 2021 Alumni Research Achievement Award winner was Nathan Gutarts.

The following recipients were chosen as winners of the 2021 Dean’s Award for Excellence in Research for their innovative projects. Nathan Gutarts, Nathan Kim, and Aakriti Chaudhry won the first-, second-, and third-place Dental Student Awards; Emily Sprinkle won the Dental Hygiene Research Award; Kazune Pax, Victoria McLaughlin, and Eun Sang Moon won the first-, second-, and third-place Graduate Student Awards; Fatma F. Mohamed received the Postdoctoral Fellow Award; and Kazune Pax won the award for the CCTS Best Clinical and Translational Abstract.*

*This award is co-sponsored by The Ohio State University Center for Clinical and Translational Science.
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Dentoalveolar Defects from Neural Crest Conditional Deletion of Bone Sialoprotein

Natalie L. Andras¹, Michael B. Chavez¹,², Michelle H. Tan¹, Tamara N. Kolli¹, Brian L. Foster¹

Objectives: Bone sialoprotein (BSP) is an extracellular matrix protein in bones and teeth. The cellular origin and associated function(s) of BSP in mineralized tissue development remain unclear. Global ablation of BSP in mice contributes to reduced acellular cementum, defective bone mineralization, and periodontal breakdown. To test tissue-specific functions of BSP, we developed a mouse carrying a floxed {Ibsp} allele (Ibspf/f). We conditionally deleted Ibsp from neural crest-derived ectomesenchyme using Wnt1-Cre2 mice. We hypothesized that BSP in ectomesenchyme contributes to cementum and alveolar bone mineralization and periodontal function.

Methods: Mandibles were harvested from Wnt1-Cre2+; Ibspf/f (cKO) and wild-type (WT; Ibspf/f) control mice (n=6/group/time point) at 30 and 90-days-post-natal (dpn). Mandibular bone and tooth development were analyzed by micro-computed tomography (micro-CT) and histology.

Results: Micro-CT revealed that enamel and dentin volumes and densities from first mandibular molars were undiminished in cKO vs. WT mice at 30 and 90 dpn. Both alveolar bone and mandibular basal bone volumes were decreased 10-20% in cKO vs. WT mice at both ages (P<0.05-0.001). Alveolar bone hypomineralization in cKO mice became relatively worse over time. Alveolar bone proper (ABP; bone closest to molar roots and involved in attachment) was dramatically reduced in both volume (40-60%; P<0.0001) and mineral density (4-6%; P<0.01-0.001) at both ages. With reduced ABP, periodontal ligament (PDL) volume of cKO mice was significantly increased for both time points. Histology revealed additional signs of periodontal defects and dysfunction, including thin acellular cementum, excess alveolar bone osteoid and cellular cementum cementoid, detachment and disorganization of the PDL, and long junctional epithelium.

Conclusions: The conditional deletion of BSP in neural crest cells contributed to decreased dentoalveolar mineralization, particularly targeting cementum and alveolar bone, and recapitulating periodontal breakdown from mice where BSP was globally ablated. These results highlight the importance of BSP in periodontal development and function.
Tgfbr2 in dental pulp cells guides sensory innervation in developing teeth

Monica Stanwick¹, Courtney Barkley², Andrew Krugel¹, Chandler Ashman¹, Kevin Nguyen², Allie Staats¹, Rosa Serra², Shifa Shahid¹, and Sarah B. Peters¹²

Transforming growth factor β (TGFβ) plays an important role in tooth morphogenesis and mineralization. During postnatal development, the dental pulp (DP) mesenchyme secretes neurotrophic factors that guide trigeminal nerve fibers into and throughout the DP. This process is tightly linked with dentin formation and mineralization. Our laboratory established a mouse model in which Tgfbr2 was conditionally deleted in DP mesenchyme using an Osterix promoter-driven Cre recombinase (Tgfbr2cko). These mice survived postnatally with significant defects in bones and teeth, including reduced mineralization and short roots. Reporter imaging demonstrated that Osterix-Cre activity was isolated to the DP mesenchyme.

We performed an mRNA-Sequence and gene ontology analysis using RNA from the DP of P7 control and mutant mice to investigate the pathways involved in Tgfbr2-mediated tooth development. These analyses identified downregulation of several mineralization and neuronal genes in the Tgfbr2cko DP compared to controls. Selected gene expression patterns were confirmed by quantitative real-time PCR. Immunofluorescent staining for a neuronal marker, β3 tubulin, was performed on serial cryosections from control and mutant molars at two different postnatal stages. Confocal imaging and pixel quantification for β3 tubulin demonstrated reduced innervation in Tgfbr2cko first molars at P7 and P24 compared to controls. Lastly, trigeminal neurons were co-cultured atop Transwell filters overlying primary Tgfbr2f/f DP cells. Tgfbr2 in the DP was deleted with Adenovirus expressing Cre recombinase.

Confocal imaging of axons through the filter pores showed increased axonal sprouting from neurons cultured in the presence of Tgfbr2-positive DP cells when compared to neurons cultured alone. Axon sprouting was reduced when Tgfbr2 was knocked down in the DP. Reduced expression of dentin sialophosphoprotein in DP cells confirmed reduced mineralization potential with Tgfbr2 deletion. These results indicate that Tgfbr2 in differentiating DP mesenchyme regulates axonal guidance during sensory innervation of developing tooth.
Streptococcus mutans Isolates Exhibit Diverse Phenotypes Dependent on Commensal Competitor

Madisen Bangs and Justin R. Kaspar

Objectives: *Streptococcus mutans* (SMU), a common dental caries pathogen, exhibits both antagonistic and synergistic relationships with other oral streptococci. Our group has a collection of SMU isolates from various locations around the globe that have previously been shown to display diverse growth phenotypes. However, behavior of the isolates during interspecies interactions has not been previously explored. In this study, we measured the biofilm formation of the isolates both in isolation as well as in competitive relationships against various oral species.

Methods: Eleven oral species, consisting of both health and disease-associated streptococci and non-streptococci, were co-cultured with twenty different SMU isolates. Both SMU isolates and selected oral species were inoculated in equal proportions into TYG (tryptone-yeast extract-glucose) medium supplemented with 5 mM sucrose in cross-like patterns within 96 well plates and incubated for 24 hours. Biomass was then quantified using the crystal violet biomass assay. Resulting data as well as simple linear regression trends were plotted and analyzed via GraphPad Prism.

Results: We found that phenotypes were dependent on the specific isolate and oral species they were co-cultured together during growth. There were no common biomass trends applicable to all co-culture interactions, indicating different levels of interspecies cooperation amongst the isolate collection. Co-cultures with disease-associated *Lactobacillus casei* displayed the most diverse array of phenotypes. For example, SMU isolate 108 exhibited robust mono-culture biofilm formation, but decreased co-culture biofilm biomass in the presence of *L. casei*. However, SMU isolate 107 had low mono-culture biomass, and biofilm formation was increased in the presence of *L. casei*.

Conclusions: After analysis, it is evident that not all isolates behave the same way in the presence of different competing oral species. This investigation reinforces the diversity of *S. mutans* isolate behaviors, and highlights the importance of studying numerous isolates rather than only a singular reference strain for the species. Moving forward, we will use fluorescent microscopy to compare specific interaction pairs that displayed high levels of variability within our assay. By studying interspecies interactions with different genomic isolates, we can gain better understanding of critical factors that drive the fitness of pathogens such as *S. mutans*.
Neuroscience Student  
Advisor: Justin R. Kaspar, PhD  
Division of Biosciences

Development of Fluorescent Microscopy Protocol for Analysis of Multi-species Oral Biofilms

Jordan Batagower and Justin R. Kaspar

Objectives: Oral streptococci develop synergistic and/or antagonistic relationships with other microbes within their niche. One example is the competitive interactions between disease-related mutans streptococci and commensal, health-associated streptococci that form biofilms using glucan polysaccharides. Fluorescent microscopy is a common technique that gives us the ability to visualize biofilm architecture and spatial arrangement which can be coupled with image quantification to assess the biomass of bacterial cells and matrix. Here, we aimed to develop a standard lab protocol that will allow us to observe and quantify individual streptococcal species within co-cultured biofilms, as well as matrix components such as glucans.

Methods: We utilized a Streptococcus mutans strain that constitutively-expressed green fluorescent protein (gfp) and commensal streptococci (Streptococcus sp. A12 and Streptococcus gordonii DL1) that constitutively-expressed dsRed-ExpressII. During bacterial inoculation, AlexaFluor 647-labeled dextran were added with the expectation it would be incorporated into synthesized glucan polymers during biofilm formation. After 24 h of growth, biofilms images were captured with a Biotek Lionheart FX wide-field fluorescent microscope using 20X magnification. The Biotek Gen5 software was used to quantify specific features of each individual fluorescent channel, such as number of microcolonies and biomass.

Results: We used our developed protocol to determine if mutant strains of both Streptococcus mutans and/or commensal streptococci altered biofilm spatial structure and accumulated biomass during co-culture between the species. In our first experiment, we inoculated S. mutans with commensal mutants of spxB, which encodes pyruvate oxidase and is responsible for hydrogen peroxide production. We observed visual and quantifiable differences in species and matrix biomass. In our second experiment we introduced mutant S. mutans to a commensal mix and identified visual and quantifiable differences as well.

Conclusions: Our fluorescent microscopy protocol is successful in the visualization and quantification of biomass changes between individual oral streptococcus species. This aids in better describing resulting changes that occur by being able to distinguish between individual species as well as biofilm matrix. Through the success of this protocol in vitro, we expect to gain more insight into what may be occurring in vivo during biofilm formation of streptococci in the oral cavity.
Neuroscience Student
Advisor: Justin R. Kaspar, PhD
Division of Biosciences

Growth Environment and its Impact on Competition Between Oral Streptococci

Paige Bending, Kevin Dong, Justin R. Kaspar

Objectives: Oral streptococci engage in both advantageous and adverse relationships with other microbes occupying the same niche. Our group has worked to identify and analyze the interspecies interactions that arise between both caries-associated mutans streptococci and commensal, health-associated streptococci. However, one predominant question remains: does the growth environment affect these exchanges between oral streptococci? To explore this question, our team compared competitive interactions between *Streptococcus mutans* and commensal oral streptococci in various growth media, including tryptone yeast extract containing glucose (TYG), and TYG supplemented with either 25% artificial saliva (AS) or human saliva (HS).

Methods: To determine if competition was altered in media containing 25% AS, we tracked 24 h growth of a *S. mutans* constitutively-active green fluorescent protein (*gfp*) reporter strain during co-culture with commensal oral streptococci using a Biotek Synergy H1 plate reader. We compared biofilm formation of *S. mutans* in TYG or TYG supplemented with either 25% AS or HS using the same *gfp* reporter strain. Fluorescent microscopy was conducted 24 h after biofilm inoculation using a Biotek Lionheart FX wide-field fluorescent microscope. We also quantified glucan matrix biomass by adding Alexaflour 647-labeled dextran during inoculation. Biotek Gen5 software was used to quantify specific biofilm features such as biomass and number of *S. mutans* microcolonies per field of view.

Results: Using the calculated area under the curve, we determined that the fluorescent intensity of *S. mutans* within co-cultures increased in TYG supplemented with 25% AS. However, microscopy showed a clear visual and quantifiable decrease in *S. mutans* biomass when grown in AS and HS compared to TYG alone. Glucan accumulation also decreased when grown in AS, but increased when grown in HS.

Conclusion: Data obtained from this study highlights the importance of investigating microbial interactions in environments close to their *in vivo* niche. Interspecies competition decreased when grown with AS compared to TYG alone. However, *S. mutans* accumulated less biomass in both HS and AS conditions. Going forward, the next step would be to observe co-cultured biofilms with AS or HS via fluorescent microscopy to understand how the oral commensal species biomass are also affected.
Identification of Genes that Impact Fitness in *Streptococcus gordonii*

Robert Bettinger and Justin R. Kaspar

**Objectives:** Oral streptococci, including the health-associated commensal *Streptococcus gordonii* and the caries-causing pathogen *Streptococcus mutans*, interact with each other within dental plaque biofilms. Gene expression of *S. gordonii* was previously measured during co-culture growth with *S. mutans* via an RNA-Seq experiment. We hypothesize that differentially-expressed genes within this dataset, particularly up-regulated genes, are critical for *S. gordonii* during interspecies interactions. However, it remains unclear how the viability of *S. gordonii* is impacted when these genes are lost. The goal of this study is to determine if mutation of selected genes affects the fitness of *S. gordonii* in competitive exchanges with *S. mutans*.

**Methods:** Four genes in *S. gordonii* were selected that were significantly up-regulated from the previous co-culture RNA-Seq data. Selected genes underwent allelic replacement with an erythromycin antibiotic marker to construct individual mutant strains. *S. gordonii* was transformed with each mutant construct using the genetic competence stimulating peptide CSP. Whole genomic sequencing of resulting clones was completed to verify the deletions, and all mutant strains contained no single nucleotide polymorphisms (SNPs) compared to the parental. Growth of the new mutant strains were assessed in different medium conditions. Crystal violet biomass assays were used to compare biofilm formation both in mono-cultures and in co-cultures with *S. mutans*.

**Results:** Through our cloning procedures, four viable mutants, Δ0396, Δ0480, Δ1862, and Δ1892, were produced and genome sequence confirmed. The growth curves of these mutants showed that all mutants, except Δ1862, maintained growth patterns similar to the parental strain. Through crystal violet biomass assays, it was found that Δ0480 had a decrease in biofilm accumulation relative to the parental strain.

**Conclusions:** The cloning of our four selected *S. gordonii* mutants was successful. The next steps are to determine how the fitness of *S. gordonii* is impacted during competitive fitness assays with *S. mutans*. By analyzing genes that may have a role in interspecies interaction, we can gain a broader understanding of how these microbes interact with each other during biofilm formation, leading to shifts that result in dental caries disease development.
Rat tooth extraction protocol for proteomics analysis of the dentin matrisome

Michelle Blyumin, Henry Fishbach, DDS, Sarah B. Peters, PhD

Objective: The overall objective of this project is to identify changes in the dentin matrisome with age. There are complications to obtaining sufficient numbers of healthy human molars at varying stages of life. Rat molars present a viable pre-clinical model of human molars with which to study dentin-aging. However, rat teeth are uniquely difficult to extract due to their diverting molar roots, multiple fused cusps, and continuous cementum deposits. In this project, we developed a protocol to isolate rat molars in young and old male rats for proteomics analysis.

Methods: First, second, and third rat molars were extracted from harvested maxillae and mandibles from young (3 months old; n=4) and old (24 months old; n=4) samples using a high-speed handpiece. The edge of an original taper fissure bur was used to thin the alveolar bone and expose the molar roots. A micro shallow taper fissure bur was used to remove bone between and around the molars to loosen them from the jaw. Then, each tooth was cleaved along the cervical line with the tip of a fissure grip needle bur. A 28-gauge syringe was used both to open the pulp chamber in the roots and to remove the dental pulp from the roots and crowns. The rat teeth will be cryogenically milled into a fine powder for subsequent extraction of dentin matrix proteins followed by proteomic analysis. Initial investigation of proteomics analysis of rodent dentin was performed with mouse molars similarly extracted.

Results: These methods successfully extracted rat molars and removed the dental pulp. Proteomics analysis was tested using milled mouse teeth. Proteomics analysis of extracted mouse molars identified several common dentin extracellular matrix proteins including dentin sialophosphoprotein, dentin matrix protein 1, and osteopontin. Proteoglycans and complement factors were also identified.

Conclusion: We developed a reproducible technique to harvest and analyze rodent dentin. Our future investigations will compare young and old rat samples. The information gained from these experiments will serve as a framework for future investigations into human tooth aging.
Impact of Growth Environment on Quorum Sensing Inhibition in *Streptococcus mutans*

Adam Bouchendouka, Michael Rose, and Justin R. Kaspar

**Objectives:** Oral streptococci interact with each other through either synergistic or antagonistic relationships in dental biofilm communities. A previous RNA-Seq showed down-regulation of *S. mutans* quorum sensing-related pathways that include both genetic competence development as well as the production of peptides that elicit cell death in target species, known as bacteriocins, in a co-culture with health-associated streptococci. However, because gene expression was only determined in TYG medium, it is unknown whether growth environment impacts the quorum-sensing phenotype observed. In this study, we assessed the production of bacteriocins and expression of the *comX* gene, the master regulator of competence development, in different growth environments through modulation of carbohydrates and their concentrations.

**Methods:** The production of bacteriocins in *S. mutans* in various co-cultures was determined using a bacteriocin overlay assay with TY agar plates that contained either glucose or galactose. The concentration of glucose was also assessed using full strength or half-strength (i.e., diluted) TYG. Activation of genetic competence in *S. mutans* was measured with a fluorescent P*comX*-gfp reporter strain, co-cultured with either *Streptococcus* sp. A12 or *Streptococcus sanguinis* with decreasing concentrations of glucose in the medium.

**Results:** Comparing glucose to galactose agar plates, we found no restoration of bacteriocin production by *S. mutans* in co-culture with the health-associated streptococci. Similarly, no difference was observed due to the change in concentration of glucose. The PcomX reporter assay indicated that regardless of the concentration of glucose, competence development was inhibited.

**Conclusions:** We found that inhibition of *S. mutans* quorum sensing-related pathways during co-culture with health-associated streptococci were not impacted by either changing of glucose concentrations or the use of galactose as the sole carbohydrate source. Therefore, we can conclude that the inhibition of quorum sensing-related gene expression during *S. mutans* co-culture is solely influenced by the presence of a health-associated streptococci species and is not dependent on growth environment. However, more conditions need to be tested to verify these results, including use of saliva. Future studies will determine whether quorum-sensing related pathways such as genetic competence influences the spatial arrangement and biofilm architecture of *S. mutans* microcolonies during interspecies interactions and biofilm formation.
Isolates of Caries Pathogen *Streptococcus sobrinus* Display Diverse Phenotypes

Timothy Dang and Justin R. Kaspar

**Objectives:** Two different species of mutants group streptococci are commonly isolated from caries lesions: *Streptococcus mutans* and *Streptococcus sobrinus*. *S. mutans* is found more frequently from diseased sites, and thus has been the main focus of oral microbiology research. However, co-occurrence of both organisms leads to more severe disease outcomes, thus the need for characterization of *S. sobrinus*. Here, our objective was to begin characterizing this species in greater detail by examining the growth phenotypes of four different *S. sobrinus* strains.

**Methods:** Growth of four *S. sobrinus* strains, 6175, K1R, SL1 and TEA, were monitored for 48 h using a Bioscreen C plate reader in the following growth medium conditions: in the complex medium TYG (tryptone-yeast extract-glucose), TYG supplemented with 25% artificial saliva, the chemically defined medium CDM, and CDM with competence-stimulating peptide XIP. Biofilm formation of the species were measured in mono-culture and co-culture against *S. mutans* and health-associated streptococci using crystal violet biomass quantification 24 h after biofilm inoculation.

**Results:** We did not find changes in growth in any of the four strains between the TYG and the TYG supplemented with mucin. However, there was a decrease in growth in TYG supplemented with 25% artificial saliva. When we grew the four strains with XIP, a quorum sensing peptide that promotes cell death due to bacteriocin production, we did find that XIP peptide was sensed by strains 6175 and K1R, but not SL1 and TEA. In the crystal violet biofilms, some strains of *S. sobrinus* showed increased biomass when co-cultured with *S. mutans*.

**Conclusions:** We observed diverse growth phenotypes when comparing our four strains of *S. sobrinus*. Data from this study present evidence of synergistic interactions of *S. sobrinus* with *S. mutans*. Looking towards the future, our objective is to further define the relationship between *S. mutans* and *S. sobrinus*. We will utilize fluorescent microscopy of co-cultured biofilms to determine specific species and matrix component biomass, as well as biofilm spatial structure changes when the two disease-related organisms are co-cultured together to gain further insight on why co-occurrence of these two organism leads to worsened disease outcomes.
A narrative review on risk assessment tools as prognostics of implant supported dental restorations

E. Dixon*, Y. Kang, and B. Leblebicioglu

Objective: Well-established risk assessment tools exist for carries and periodontal disease. Similar tools to determine the risk of peri-implant tissue breakdown are still in the exploratory phase. This study aims to review the evidence of various site and patient specific factors as possible risks for peri-implant supported dental restoration failures with the ultimate goal of designing a clinical risk assessment tool for peri-implant diseases.

Material and Methods: Research focus questions were constructed in accordance with PICO criteria. A comprehensive narrative search in MEDLINE via PubMed database was conducted. Three reviewers screened the reports based on PICO and inclusion/exclusion criteria specific for this study.

Results: Most of the available risk assessment tools designed for implant supported dental restorations incorporate site specific factors. History of periodontal disease and smoking status are patient specific factors commonly considered as part of risk assessment. Other conditions such as metabolic syndrome, obesity, osteoporosis, hyperlipidemia, age-dependent hormonal changes are currently under investigation. Routine uptake of medications such as statins, antihypertensive medications, SSRIs and their effect on peri-implant tissue stability have been explored. Assessment of risk in relation to biomaterial/host interactions has been recently initiated in implant dentistry.

Conclusion: Risk assessment tools as prognostics of implant supported dental restorations are limited. There is a significant need to improve existing tools by incorporating biomaterial integrity and patient specific factors in parallel to increasing evidence.
Kelly Doan, Stella Kim, Do-Gyoon Kim

Objectives: Clinical and epidemiological studies have shown a higher risk of tooth loss with aging in women than men. Postmenopausal osteoporosis also shows a higher risk of decrease in bone mineral density (BMD) and bone loss in orthopedic bones of women but it has not been evaluated extensively in jawbones. The objective of this study was to examine whether the oral BMD changes differently between men and women with aging.

Methods: Following IRB approval, cone beam computed tomography (CBCT) images were retrospectively obtained from 78 patients. Thirty-seven males (15 to 70 years) and 41 females (16 to 70 years) were assigned to three age groups, including 40-age group (20 to 49 years), 50-age group (50 to 59 years), and 60-age group (older than 60 years). After bone voxels in the CBCT images were segmented from non-bone voxels, mandibular left first premolars were digitally removed from the CBCT images. Alveolar bone (AB) regions surrounding the tooth roots and basal cortical bone (CB) regions below the teeth were then digitally isolated. CT attenuation value (gray value) of each bone voxel, which is proportional to BMD, was obtained. One way analysis of variance and Pearson’s correlations were tested for aging effects on mean gray values.

Results: The mean gray values of the AB and CB regions were not significantly different between age groups for both male (p>0.33) and female (p>0.46) groups. However, the AB region of the female group had a significantly negative correlation of the mean gray value with age (p=0.04) but all other correlations were not significant (p>0.14).

Conclusions: The current result showed that BMD of AB is decreasing as women age. As AB is directly surrounding the teeth, this finding suggests a potential mechanism of the higher risk of tooth loss in women with aging.
Growth Environment Impacts Interspecies Interactions Between Oral Streptococci

Kevin Dong, Paige Bending, and Justin R. Kaspar

Objectives: Oral streptococci form both synergistic and antagonistic relationships with other microbes that occupy the same niche. Recently, our group has identified and detailed competitive interactions that occur between caries-associated mutans streptococci and commensal, health-associated streptococci. However, the role of environment during these exchanges remains undefined. In this study, we compared competitive interactions between Streptococcus mutans and commensal oral streptococci in a common lab-based growth medium (tryptone yeast extract containing glucose; TYG), and medium supplemented with a commercially-available artificial saliva that contained mucin.

Methods: The growth of eight different oral streptococci species, including both health- and disease-related streptococci, were compared in TYG against TYG supplemented with 25% artificial saliva (AS). Biofilm formation of the species were also measured in mono-culture and in co-culture competitions against S. mutans. Whole transcriptome profiling (RNA-Seq) was utilized to determine gene expression changes between the growth conditions.

Results: We found an increase in growth rates for a majority of the species growing in TYG supplemented with AS compared to TYG alone. However, TYG with AS reduced biofilm biomass after 24 h, and significantly reduced competitiveness of commensal, health-associated streptococci against S. mutans. By evaluating specific components of the AS, we found that the presence of mucins to be responsible for the majority of phenotypes displayed. Finally, we found the transcriptomes of both S. mutans and Streptococcus mitis were altered in the presence of AS, impacting the interactions between the species during growth in a co-culture mix.

Conclusions: Data obtained from this study present evidence for mucin-specific effects in biofilm formation, growth, and interactions between oral streptococci. This investigation highlights both the importance of evaluating microbial interactions in growth environments that more closely mimic their natural niche, and the potential role of host factors (mucins). By studying intermicrobial interactions, we can gain broader understanding of critical factors that induce ecological shifts in microbiome composition and behaviors.
Cervical Vertebral Bone Density Changes in Women with Aging

Brandon Graf, Eun-Sang Moon¹, Do-Gyoon Kim¹

Objectives: Postmenopausal osteoporosis is a systematic bone disease in women with aging. Most of the osteoporosis diagnosis has been conducted by assessing bone mineral density (BMD) of thoracic or lumbar spine using 2 dimensional image of dual energy X-ray absorptiometry (DXA). However, recent clinical observations showed that prevalence of aging related cervical vertebral injury considerably increases. Thus, the objective of the current study is to examine whether dental 3-dimensional cone beam computed tomography (CBCT) is applicable to assess BMD of the cervical vertebrae.

Methods: Following IRB approval, 143 CBCT images were retrospectively obtained from 54 male and 89 female patients (6 to 84 years of age) on routine dental patients. The CBCT images were assigned for 3 age groups including 40-age group (20 to 49 years), 50-age group (50 to 59 years old), and 60-age group (older than 60 years old). The second cervical vertebral body (C2) were digitally isolated by removing posterior and lateral processes. A gray value, which is proportional to BMD, was assessed. Mean gray values of the C2 were computed and tested using one way analysis of variance between age groups and Pearson’s correlations with age. Significance was set at 0.05.

Results: The female 60-age group had a significantly lower mean gray value than female 40 age group (p=0.003) while it was not significantly different between all other age groups in male and female groups (p>0.09). The female group showed a significant negative correlation of the mean gray value with age (p<0.001) but it was not significant for the male group (p=0.188).
Objective: Estrogen deficiency induces postmenopausal osteoporosis decreasing tissue mineral density (TMD) in orthopedic bone. However, it is controversial whether jawbone is also altered. Thus, the objective of this study was to examine the effect of estrogen deficiency on TMD distribution in jawbone using ovariectomized (OVX) rat model.

Methods: After approval of IACUC protocol, 13 female Sprague Dawley rats including 10 wild type (WT) (9 months old) and 4 OVX (3 months following OVX surgery at 6 months old) rats were obtained. Their mandibles were dissected and scanned using micro-computed tomography (micro-CT) with 20 micron voxel size. Hydroxyapatite phantoms of known density values were used to calibrate the CT attenuation values of the voxels to TMD. Bone voxels were segmented from non-bone voxels and teeth were digitally removed. Histograms of TMD were obtained to determine mean, standard deviation (SD), fifth percentile low (Low5) and high (High5) TMD for each mandible. One way analysis of variance was performed to compare the TMD parameter values between WT and OVX groups and Pearson’s correlation was also tested. A significant level was set at p<0.05.

Results: All TMD parameters were significantly higher for the OVX group than the WT group (p<0.02). The WT group had strong positive correlations of the mean TMD with all other parameters (p<0.002) while the OVX group had a significant correlation only between the mean TMD and High5 (p=0.014).

Conclusion: The current findings showed that jawbone is less sensitive to estrogen deficiency rather its tissue mineral contents increases. The High5, which is altered with removal of pre-existing bone tissues, may control the mean TMD of OVX rat by estrogen deficiency induced bone resorption.
Dental crowding predicted by machine learning from clinical photos

Parker Heiner, Tai-Hsien Wu, Ching-Chang Ko

Objective This study aims to determine the feasibility of machine learning in analyzing maxillary dental crowding using clinical intraoral photos.

Background Research on machine learning in orthodontics has been growing in the spaces of treatment planning, diagnosis, and solving controversial questions in orthodontics such as extraction vs. non-extraction. Current assessments use manual measurement via models and digital calipers. However, the traditional method is highly empirical, and the results vary among clinicians. Since convolutional neural network (CNN) models have been successfully used for automatic medical image classification for disease diagnosis, this study applies two classic CNNs on the prediction of maxillary crowding using only intra-oral maxillary images as input.

Methods Maxillary crowding was previously measured by orthodontic residents on 835 patients and maxillary intraoral photos were taken. Two classic CNN models, Visual geometry group (VGG) and Residual neural network (Resnet), were trained and validated on 668 of the maxillary images. The well-trained models then predicted the crowding values on the remaining 167 images, and these predictions were compared to the clinical measurements of crowding.

Results The Resnet model performed better than the VGG model in approximating the clinical measurements of crowding. Of the 167 test images, the Resnet model returned 141 (84%) measurements with an error ≤3 mm, 13 (8%) measurements with an error >3 and ≤6 mm, 6 (4%) measurements with an error >6 and ≤9 mm, and 7 (4%) measurements with an error >9 mm.

Conclusions The Resnet model applying CNN to measure crowding of images is promising. Future research will use landmark annotation and exclusion of confounding areas of the image to reduce prediction errors. This study’s fundamental work suggests CNN’s capability to learn orthodontic features, which may improve workflow efficiency in orthodontic treatment and education.
Objective: Previous studies have shown evident changes in tooth structure and health as early as age 40, but there is a gap in knowledge in how to maintain tooth vitality with age. On an esthetic level, teeth are important for appearance, influencing public, professional and personal lives of humans. On a functional level, teeth are used for speech, cutting and chewing food, and parafunctional habits such as grinding and clenching. The daily wear from these activities leads to an expected decline in their physical components. Teeth are primarily composed of dentin, which contains an extracellular matrix that is mineralized with hydroxyapatite crystals. This dentin encases a soft, mesenchymal pulp tissue that contains neuronal projections and vasculature. The odontoblasts at the pulp-dentin interface deposit secondary dentin throughout the life of the tooth and reactionary or reparative dentin in instances of damage and/or infection. With age, this reduces the pulp chamber volume and thereby reduces the odontoblast, neuronal, and immune components and their ability to respond to insult and infection. It remains unknown how ongoing mineralization and the resultant pulp-dentin morphological alterations affect tooth vitality. The objective of this study is to confirm age-related pulp-dentin changes reported in human teeth using the mouse model.

Methods: We performed micro-computed tomography at a 10 µm resolution on 3 and 27 month old male WT mice, which are the human equivalents to 20-30 year old and 70+ year old men, respectively. The pulp, dentin, and enamel volumes were measured, as well as mean mineral density using histograms.

Results: Micro CT confirmed a decrease in dental pulp volume and increase in mineral density as mice age.

Conclusions: These results demonstrate a reduction in dental pulp volume and increase in dentin density with age. Future investigations will investigate surgically induced tooth injuries in aging rodent teeth to determine if dentin composition plays a role in injury responses and long-term tooth vitality.
Gingival Epithelium Gene Regulation and Inflammatory Response to JUUL E-cigarettes

Joe Kainrad, Michelle L Scott, Shareef M Dabdoub, Shinae Kim, Purnima S Kumar

Objectives: Electronic cigarettes, such as JUUL, have become a massively popular alternative to tobacco cigarettes in the United States. Additionally, the percentage of younger individuals using JUUL has increased dramatically over the years. This investigation intended to discover the effects of JUUL on subgingival oral host-microbial interactions.

Methods: To mimic the oral subgingival environment, multispecies microbial communities were created in vitro on sterilized hydroxyapatite discs. Colonization was simulated by adding six pioneer species, followed by *Fusobacterium nucleatum*, and eight tertiary colonizers 24 hours later to act as the primary, secondary, and tertiary biofilms respectively. Biofilms were placed onto Epigingival organoid tissues to create a mucosal interface. Samples were exposed to either clean air, 3% or 5% nicotine “Virginia Tobacco” JUUL. Expression of 8 pro- and anti-inflammatory cytokines was measured via multiplexed bead-based flow cytometry. RNA isolation of the host epithelial tissues was completed, followed by mRNA enrichment, sequencing, and alignment. Annotation of differentially expressed genes between control and JUUL groups was completed via PANTHER classification system.

Results: RNA analysis resulted in significant (p<0.05, FDR) differences between the 5% nicotine JUUL and control samples in the secondary biofilm samples. 10 genes showed significant upregulation, while 305 showed significant downregulation. When comparing secondary biofilms to primary and biofilm-free samples, there was a significant dampening of the pro-inflammatory response. Key inflammatory mediators, interferon-gamma and tumor necrosis factor alpha, were significantly decreased when comparing secondary biofilm exposure to 5% JUUL and controls.

Conclusions: JUUL aerosol may contribute to a dampened inflammatory response to pathogenic bacteria. This may predispose certain individuals to periodontitis as pathogenic expansion would be far more accessible.
Sex and Tooth Site Dependent Bone Mineral Density with Aging

S. Kim, M. Hwang, I. Huh, S. Kim, D. Kim

**Objectives:** Bone mineral density (BMD) surrounding teeth varies to bear a masticatory loading dependent on their functional demand. It is suspicious that elderly women show a higher risk of tooth loss because of reducing more oral BMD with aging than men. Thus, the objective of the study was to examine sex and tooth site dependent changes of oral BMD with aging.

**Methods:** IRB was approved for analysis of 84 clinical cone beam computerized tomography (CBCT) images composed of 42 female and 42 male patients (age range of 10 to 60 years). An imaging software (ITK Snap) was used to separate bone voxels from non-bone voxels. The alveolar bone (AB) regions of incisors and left first premolar in the mandibular arch were isolated within 1 mm outside the root surface. The basal cortical bone (CB) regions of identical dentition was measured at 0.6 mm inside the mandibular bone borders. The mean gray level, which is proportional to bone mineral density (BMD), was measured for each bone regions. Pearson’s correlation was tested for the mean gray levels of AB and CB with aging at each tooth site of male and female groups.

**Results:** The female group had a significant negative correlation of the mean gray value with age at the AB of incisor region (p=0.028) while no correlations were found for all other regions in female and male groups (p>0.05).

**Conclusions:** The current findings give an insight that the mandibular incisor of female group may have the higher risk of tooth loss with aging because of the significant age-dependent reduction of AB BMD surrounding the tooth.
Facial Morphology Associates with Bone Mineral Density at Mandibular Condyle

Nathan Kim1, Paul J. Kim1, Christine H. Lee1, Minji Kim1, Do-Gyoon Kim1

Introduction: Temporomandibular joint (TMJ) is a synovial joint that hinges the mandibular condyle to the articular eminence of the temporal bone. The TMJ osteoarthritis (TMJ OA) is a degenerative disease that exhibits alterations of articular cartilage, and subchondral and trabecular bone resulting in changes of bone mineral density (BMD). Women show a higher prevalence of TMJ OA than men. The objective of this study was to examine whether facial morphology is associated with BMD of the mandibular condyle.

Methods: Following IRB approval, clinical cone beam computed tomography (CBCT) images of 33 male (14 to 62 years of age) and 46 female (8 to 64 years of age) patients were retrospectively examined. Right and left mandibular condyles were digitally isolated from CBCT images and bone voxels were segmented from non-bone voxels by the use of imaging analysis software (ITK-snap). Histograms of gray value, which is proportion to BMD, was obtained for each voxel. Cephalometric images were obtained from the CBCT images to analyze the mandibular morphology. Pearson’s correlations were tested for the BMD and morphological parameters. Significant level was set at p<0.05.

Results: Female group had a significantly higher mean gray value (BMD) but shorter mandibular length and height than male group (p<0.002). Only the right mandibular condyle of female group showed a significant negative correlation between the mean gray value and mandibular height (p=0.002).

Conclusion: The current findings indicate that the mandibular length is likely associated with structural stability of TMJ motion altering the BMD of the mandibular condyle of the female group.
Sex Dependent Associations of Bone Mineral Density Distribution with Temporomandibular Osteoarthritis

Paul J. Kim1, Zachary Skabelund1, Sonya Kalim2, Christine H. Lee1, Nathan Kim1, Kristen Nguyen1, Hany Emam3, Lisa Knobloch4, Toru Deguchi1, Minji Kim5, Do-Gyoon Kim1*

Temporomandibular joint osteoarthritis (TMJ OA) is a degenerative bone disease that have been observed more in women than men. However, the causes of TMJ OA are multifactorial in nature and its diagnostic criteria are highly controversial. Recently, researchers have suggested using cone beam computed tomography (CBCT) to diagnose TMJ OA by counting destructive changes of TMJ condyle. The goal of this research is to investigate whether parameters of bone mineral density distribution in the TMJ condyle and mandibular facial morphology have association with TMJ OA counts using CBCT images.

Methods: Following IRB approval, CBCT images of 35 adult patients (16 male and 19 female) between 20 and 50 years old were retrospectively examined. Experienced clinicians including a dental radiologist examined nine surfaces of the condyles to count TMJ OA changes. Right and left mandibular condyles were digitally cropped to yield bone mineral density (BMD) distribution. A histogram of gray level that is proportional to BMD was obtained for each TMJ condyle. Mean, standard deviation (SD), fifth percentile low and high values (Low5 and High5) of the gray level histogram were determined. Cephalometric images were extracted from CBCT images to measure the mandibular morphology. A two-way analysis of variance (ANOVA) was done between sex and sides of mandibular condyle to compare volume, gray value parameters, and TMJ OA counts while paired t-test was used to analyze those parameters between right and left mandibular condyle. One-way ANOVA was conducted to see effects of sex on all parameters. Pearson’s correlation coefficients were utilized to find correlations of the TMJ OA total counts with the gray value and cephalogram parameters, and between the gray value and cephalogram parameters. Significance was all set at p<0.05. (too long? For stats?)

Results: Female group had significantly higher values of mean and SD at the right mandibular condyle, High5 at both sides, and all gray value parameters for total (right +left) compared to male group (p<0.05). TMJ OA counts showed negative correlation with volume (p=0.005) and mean, Low5, High5. Specifically, antero-lateral (AL) region TMJ OA count showed significant interaction gray value parameters (p<0.05). No correlations were found between TMJ OA with SD, mandibular length, or mandibular height.

Conclusion: Female has more frequent count of TMJ OA changes in mandibular condyle compared to male and bone mineral density distribution confirmed its trend with significant positive correlations of gray value parameters. Mandibular length or height, although different between male and female, does not seem to be related to the differences in TMJ OA count. The gray value parameters of the condyles may provide useful information in patients with CBCT readings for TMJ OA risks especially when AL region of the condyle is captured.
Characterization of somatostatin neurons in the rostral nucleus of the solitary tract

Charlotte Klimovich, Sidney Li, Susan Travers

**Background:** Taste stimuli are recognized by dedicated taste receptor cells in taste buds located within the oral cavity. These receptor cells transfer information via nerves to reach the brain taste relay center, the rostral portion of the nucleus of the solitary tract (rNST), through which the signal travels on the way to its eventual destination of the taste cortex. Excitatory and inhibitory neurons in the rNST function to process the taste signal before its transmittance to other areas of the brain controlling behavioral responses to the stimuli. Our lab has established the prominent role that rNST inhibitory neurons play in controlling the taste-driven licking behaviors of mice in response to aversive stimuli like bitter-tasting quinine. A recent article by Jin et al. 2021 further examined responses to bitter taste and found a group of rNST neurons defined by expression of the neuropeptide somatostatin (Sst) that exclusively responded to bitter stimuli. Elucidating the ratio of inhibitory to excitatory cells within this group of neurons will improve understanding of how the brain processes information that indicates a food item has gone bad.

**Objective:** Our objective was to determine the excitatory or inhibitory function of the Sst positive neurons within the rNST. We utilized fluorescent in situ hybridization to label the mRNA of the vesicular transporter VGAT (a marker for inhibitory neurons), Sst, and the vesicular transporter VGlut2 (a marker for excitatory neurons). Double labeling of Sst cells with either VGAT or VGlut2 would enable identification of excitatory versus inhibitory Sst neurons.

**Methods:** Two C57BL/6J mice (Jackson Laboratory Strain #000664) were perfused with phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA) and their brains extracted. Tissue was postfixed overnight in 4% PFA in a 30% sucrose solution at 4°C and embedded in O.C.T. Compound (Tissue-Tek®) to be sectioned at 15µm on a cryostat. Coronal sections chosen from the rNST (N=4) underwent fluorescent in situ hybridization using an RNAscope® Multiplex Fluorescent V2 Assay. After the reaction was complete, slides were coverslipped and photographed on an Olympus IX83 confocal microscope at 20X and 60X.

**Results:** The resulting images of labeled cells were quantified using the Cell Counter plugin of ImageJ (Fiji), identifying 249 Sst-positive cells in total. Of these, 162 were also labeled with VGAT while 81 were also labeled with VGlut2, meaning that the ratio of inhibitory cells to excitatory cells in the rNST was about 2:1.

**Conclusion:** The predominance of inhibitory Sst neurons in the rNST presumably accounts for the observed behavioral response to aversive bitter stimuli. Our results suggest that the response to these stimuli relies on inhibitory Sst neuron modulation. Future investigations will explore the distribution and the functional role of Sst neurons across the rNST.
Malalignment factors related to the Invisalign treatment time using artificial intelligence

Sanghee Lee

Objectives: The aim of this study is to identify what type of and how the severity of the malocclusion affect total Invisalign treatment duration based on intraoral digital scan utilizing Artificial Intelligence. We hypothesize that types of tooth movement and the degree of malalignment can determine total Invisalign treatment time.

Methods: The subject of this retrospective clinical cohort are the 130 patients treated with Invisalign appliance at the Ohio state university, graduate orthodontic clinic. The initial (IM) and final (FM) 3D digital models were collected. Deep learning algorithms were developed for automatic tooth segmentation and landmark identification. The six degrees of freedom (DOF), representing types of malalignment of each tooth, were measured. Linear regression was performed to find the contributing factor associated with treatment time. In addition, the PAR score and a composite score combining 6 DOFs were separately correlated to the treatment time.

Results: AI allows massive calculation of DOF dentition with high accuracy (inter-evaluator reliability test>0.99). The individual DOF showed weak or no association (P=0.1416), while the composite score revealed a moderate to strong correlation with the treatment time (P<0.001). Pre-treatment lower anterior segment PAR score is positively associated with the treatment time. There is an evidence that the number of trays may differ between male and female. (p=0.0005)

Conclusion: A combined DOF seems to be a better predictor for total Invisalign treatment time than individual malalignment factor. The lower anterior malalignment factors seem to have more significant effect on the total treatment duration than those on upper anterior segment. Males have extended treatment duration than females while their tooth movement were not significantly different.
Alpl ablation in neural crest lineages causes dentoalveolar defects characteristic of hypophosphatasia

F.F. Mohamed1, M.H. Tan1, J.L. Millán2, B. L. Foster1

Objectives: Hypophosphatasia (HPP) is caused by loss-of-function mutations in the ALPL gene encoding tissue-nonspecific alkaline phosphatase (TNAP). HPP leads to rickets and osteomalacia, and a spectrum of dental disorders including enamel defects, thin and/or hypomineralized dentin, and premature tooth loss due to acellular cementum hypoplasia. Though Alpl global knockout (Alpl-/−) mice recapitulate severe HPP and have been instrumental for studying disease mechanisms, their early lethality limits studies of TNAP specific function(s) in dentoalveolar tissues.

Methods: We generated Wnt1-Cre2+/Alplf/f conditional knockout (cKO) mice to target Alpl deletion in neural crest (NC) cells that contribute to craniofacial structures, including teeth. We analyzed cKO and control (CTR; Alplf/f) tissues at 60 days postnatal by serum biochemistry, micro-computed tomography, and histology (n=6/genotype).

Results: In contrast to vitamin B6-dependent seizures and early lethality in Alpl-/− mice, cKO mice fed normal chow did not exhibit seizures, survived to the study endpoint, and had similar body weight vs. CTR. Compared to CTR, cKO mice showed 60% reduction in serum TNAP (p<0.01), with no changes in calcium or phosphorous levels. Compared to CTR first molars (M1), cKO mice had significantly reduced dentin volume, increased pulp volume, reduced alveolar bone volume and density, and reduced cellular cementum density (P<0.05 for all). No differences were observed in M1 enamel volume or density between cKO and control mice. Interestingly, cKO mice showed severely disturbed incisor formation with more than 50% reduced enamel volume (P<0.05) and 20% reduced dentin volume (P<0.01), compared to CTR. Histology of M1 revealed wide predentin, lack of acellular cementum, loss of periodontal attachment, and osteoid in alveolar bone in cKO vs. CTR.

Conclusions: Alpl ablation in NC-derived tissues recapitulated dentoalveolar defects of HPP, suggesting the requirement of TNAP for proper mineralization by ectomesenchymal NC lineages and providing a model to further study dentoalveolar defects of HPP and novel treatments.

Funding: NIDCR grants R03DE028411 (BF) and R01DE128889 (JLM)
Ohioan’s Preferences during COVID-19 Pandemic

Molly Nash and Taylar Rowe

**Purpose:** The purpose of this study was to investigate Ohioan’s preferences related to sources of COVID-19 information, vaccine, and teledentistry.

**Methods:** An IRB-approved, self-administered on-line survey was conducted through REDCap and launched via Ohio ResearchMatch in October of 2021 asking participants about their preferences related to sources of COVID-19 information, vaccine, and teledentistry.

**Results:** Fifty-three individuals completed the survey. Majority of participants were over age 35 (82%), female (83%), and white (92%). Regarding where participants obtained most of their information about COVID-19, 25% indicated the news, 25% from on-line sources, 23% from research articles, 16% from their doctor, 8% from other sources and 2% from friends/family. Majority (84%) reported they will not get the COVID-19 vaccine when it is offered. When asked about willingness to get the vaccine if there was an associated cost, 78% indicated they would not be willing to do so. Less than half (45%) of participants reported having received the flu shot this year. Related to teledentistry, 72% reported they would be interested in online consultation and screening prior to the dental appointment to assess dental needs. Given no barriers with access to a computer and internet, 96% reported they would not be willing to fill out health history, dental history forms, and insurance questions electronically instead of completing them in the office.

**Conclusion:** Overall, there was a negative view toward COVID-19 vaccine and a positive view toward using teledentistry. Participants obtained most of their information about COVID-19 through news or online sources.
Peri-implant Titanium Content in Relation to Clinical Health

Nguyen V, Kandaswamy E, Sakulpaptong W, Powell H, Tatakis DN, Leblebicioglu B

Objectives: This study aimed to determine peri-implant fluid titanium (Ti) content during early wound healing. Working hypothesis is that Ti contamination is initiated during implant fixture placement. Methods: Test group (T): patients scheduled for their first dental implant placement surgery. Control group (C): subjects with clinically healthy implant supported crowns, in function ≥1 year. For T, wound fluid (WF) and saliva were collected prior to surgery, at 10, 30, and 120 days post-operatively. For C, peri-implant crevicular fluid (PICF) was sampled. Saliva, WF, and PICF samples, stored in -20°C, were processed in nitric acid with trace amounts of hydrofluoric acid until complete digestion. Extracts were analyzed for Ti using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Half of the WF and PICF samples were used to detect Ti while the remaining half were used to determine inflammatory cytokine content (multiplex assays).

Results: T group: 16 patients have been recruited (aged 44±3 years; 10 female), 8 sites (50%) were pristine bone and 9 implants (56%) were placed with two-stage protocol. Ten cases (63%) were replacement of a maxillary tooth with 5 cases (31%) replacing missing maxillary anterior tooth. C group: 18 subjects (61±4 years; 12 female; 8±2 years in function) were included, with 9 maxillary and 9 mandibular implants, 83% replacing a posterior tooth. In C group, Ti was detected in 15 PICF samples (83%; 12.4±3 ppb) with high IL-8 and IL-1β levels (53,593±13,839 and 4,161±915 pg/ml, respectively) and low IL-4 levels (4.6±1 pg/ml). A trend of increased IL-4 levels was noted with increasing Ti levels (p>0.05). Experiments and data analysis for T group are ongoing.

Conclusion: PICF Ti contamination is a common finding around restored dental implants diagnosed with clinical health.

Support: a grant from Osseointegration Foundation to BL and by Advanced Training in Periodontics Program at OSU
Tracking Sources of the Placental-Microbiome in Normal and Pre-term Births

Kazune Pax, Murat Alan, Pinar Meric, Onder Gurlek, Nurcan Buduneli, Shareef Dabdoub, Purnima Kumar

Objectives: In the US, pre-term birth affects 1 in 9 babies and pre-eclampsia occurs in 3-8% of pregnancies. While the cause of pre-eclampsia is unknown, placental dysfunction caused by angiogenic imbalances and inflammatory disturbances plays a role. Dysbiosis in the microbiome may be a factor in inflammation that leads to adverse pregnancy outcomes. We therefore aimed to investigate whether the oral microbiome has a role in contributing to pre-term delivery or pre-eclampsia.

Methods: Saliva, plaque, serum, and placental samples were collected from 130 women (45 healthy, 36 with pre-eclampsia, and 49 who delivered pre-term). DNA was isolated and underwent whole genome sequencing. Taxonomy was assigned using Kraken against the Human Oral Microbiome Database (HOMD). Sequences were functionally annotated against the KEGG, CARD, and VFDB databases. The heterogeneity of the microbiome among the 130 women was measured using a beta-dispersion index. SourceTracker quantified the contributions of the salivary, plaque, and serum biomes to the placental biome.

Results: All placental samples in the study demonstrated a microbiome and did not differ between vaginal or c-section (p-value=0.973, Aitchison distance, PERMANOVA). However, functionally, placental samples demonstrated significant beta dispersion in all three groups. This was not observed in sequences of salivary or serum origin. Aitchison distance revealed clustering of the placental microbiome based on pregnancy outcome, both taxonomically and functionally (p-value=0.001 for both, PERMANOVA). SourceTracker revealed that saliva was the predominant source of microorganisms and functions in serum in all three groups. SourceTracker also revealed serum to be the predominant source of the placental biome. In mothers with pre-eclampsia, saliva and plaque was an additional source of the placenta’s biome.

Conclusion: The oral microbiome is a source of the placental microbiome and the translocation of certain oral species to the placenta via the serum is associated with pre-term delivery and pre-eclampsia.
Oral Biology PhD Candidate
Advisor: Dr. Brian Foster

Division of Biosciences; University of Iowa College of Dentistry; Department of Prosthodontics and Periodontics/Division of Periodontics (State University of Campinas, Sao Paulo, Brazil); Faculty of Dentistry (N. Sra do Patrocinio University Center, Itu, Sao Paulo, Brazil)

Effect of Sclerostin Deletion on Dentoalveolar Development and Molar Super-Eruption

Aonjittra Phanrungsuwan¹, Michael B. Chavez¹,², Michelle H. Tan¹, Tamara N. Kolli¹, Fatma F. Mohamed ¹, Cristiane R. Salmon³,⁴, Francisco H. Nociti, Jr.³,⁵, Brian L. Foster¹

Objectives: Sclerostin is encoded by the Sost gene and regulates Wnt signaling. Sclerostin is expressed by osteocytes, odontoblasts, and cementocytes. Osteocyte sclerostin expression regulates bone remodeling, and loss-of-function of sclerostin in mice (Sost-/-) was reported to cause bone and cellular cementum overgrowth. We aimed to analyze developmental effects of Sost ablation on dentoalveolar tissues and test whether the challenge model of unopposed super-eruption promoted increased alveolar bone and cellular cementum production in Sost-/- mice. We hypothesized increased and more responsive alveolar bone and cellular cementum in the absence of Sost.

Methods: Dentoalveolar development in Sost-/- and wild-type (WT) mice was analyzed at 6 weeks-of-age. Maxillary first molars were bilaterally extracted at 6 weeks-of-age and the effect of super-eruption of the mandibular first molar was tested 21 days post-procedure (dpp) (n=3- 5/group). Tissues were analyzed using micro-computed tomography and histology (H&E and picrosirius red staining).

Results: At 6 weeks, Sost-/- and WT mice showed no differences in first molar enamel, dentin, alveolar bone, or cellular cementum volumes, though mandibular basal bone was increased 30% compared to WT. Alveolar bone proper (ABP; bone closest to molar roots and involved in attachment) volume was increased 45% and periodontal ligament (PDL) volume was reduced 20% in Sost-/- vs. WT mice, indicating altered periodontal structures. At 21 dpp, alveolar bone was dramatically increased more than 50% in Sost-/- mice, with increased ABP and reduced PDL compared to WT controls. After super-eruption, cellular cementum volume nearly doubled in WT but increased only about 30% in Sost-/- molars, therefore cellular cementum volume was substantially reduced in in Sost-/- vs. WT mice at this stage.

Conclusions: Sclerostin regulates periodontal tissue development in mice, primarily targeting alveolar bone, which was very responsive to molar super-eruption. Cellular cementum showed no developmental overgrowth at this age, and was less responsive than alveolar bone to supereruption.

Funding: National Institute for Dental and Craniofacial Research (NIDCR) grant R03 DE028632
Ohio Adults’ Perceptions and Attitudes toward Dental Visits Post-COVID

T. Rowe, M. Nash, and H. Amini

Objectives: The purpose of this study was to investigate Ohio adults’ perceptions and attitudes toward safety of visiting dental facilities post-COVID outbreak.

Methods: An IRB-approved, self-administered online survey was conducted through REDCap and launched in October of 2021 via ResearchMatch asking participants about their general feelings toward their safety and the precautions taken at their dental office.

Results: Fifty-three individuals completed the survey. Majority of participants were over age 35 (82%), female (83%), white (92%) and covered by government-funded insurance (80%). While 61% reported feeling comfortable visiting the dentist during COVID pandemic, 14% reported they will only visit the dentist if they have an emergency/pain. Many (70%) felt visiting the dental office was not risk-free. Only 2% reported being afraid of going to the dentist due to fear of contracting COVID at the dental office. Most (85%) reported having had a dental visit during the pandemic. Out of those with a reported history of dental visit, 90% felt not enough precautions were taken by the dental office to protect them from COVID-19; 74% expressed safety measures such as temperature taking, screening questions, social distancing, face shields, N95 masks, gowns, and high-vacuum suction machines would make them feel safe. Additionally, 14% indicated they would like to see more precautions beyond indicated safety measures. Interestingly, 72% felt these safety measures should not continue after the pandemic has ended.

Conclusions: Overall, participants felt comfortable with visiting dental office, while acknowledging risks involved. The more safety measures in place, the more comfortable participants felt about their dental visit.
Dynamic Mechanical Analysis in situ Mechanical Stability of Teeth

Dr. Do-Gyoon Kim

Objectives: Mechanical stability of teeth is of important in bearing high impact static occlusion and dynamic mastication. As teeth are supported by viscoelastic periodontal ligament (PDL) and surrounding alveolar bone (AB), their response to loading may be altered by bone disease including osteoporosis. It is well known that postmenopausal osteoporosis arises from estrogen deficiency. Thus, the objective of this study was to investigate whether the estrogen deficiency changes static and dynamic elastic and viscoelastic stability of teeth in situ using dynamic mechanical analysis (DMA).

Methods: Following IACUC approval, 4 groups of 27 Sprague Dawley rats including 10 wild type (9 months old), 11 bilateral ovariectomized (OVX) (4 of 9 months old at 3 months of post-OVX and 7 of 8 months old at 2 months of post-OVX) and 6 sham surgery (8 months old) were obtained. Mandibles were dissected and a hemi-mandible of each rat was mounted on a loading machine and subjected to DMA. A non-destructive compressive static displacement was applied on molars up to 0.02 mm followed by cyclic loading (-5±4N at 0.5 to 3 Hz). Static elastic stiffness (K) and hysteresis (W), and dynamic complex stiffness (K*) and tangent delta (energy dissipation ability) were measured. The values were tested using one way analysis of variance between groups and Pearson’s correlations. Significance was set at 0.05.

Result: All values were not significantly different between groups (p>0.33). A significant negative correlation was found between K and W independent of the groups (p=0.02).

Conclusion: The DMA successfully assessed elastic and viscoelastic behavior of teeth in PDL and AB socket. The current finding showed that estrogen deficiency had not significant effects on the mechanical stability of teeth. Increase in elastic stiffness may compromise less energy dissipation ability to maintain stability of teeth under static loading.
Impact of JUUL e-cigarettes on oral host microbial interactions

Michelle L Scott, Prem Preshant Chaudhary, Shareef M Dabdoub, Joe Kainrad, Purnima S Kumar

Objectives: Emerging evidence implicates e-cigarettes, especially JUUL, in the disruption of host microbial homeostasis in several ecosystems. Therefore, the present study investigated the effects of JUUL on oral host-microbial interactions using a combined multi-omics approach.

Methods: *In vitro* microcosm communities were created by seeding six pioneer species, followed by *Fusobacterium nucleatum*, followed by eight tertiary colonizers on sterilized hydroxyapatite discs to create primary, secondary and tertiary biofilms, respectively. Biofilms were overlayed onto Epigingival organoid tissues. The constructs were exposed to clean air or “Virginia Tobacco” favored JUUL with 3% or 5% nicotine. Tissue and biofilm RNA were sequenced and annotated to the PANTHER and KEGG databases, respectively. Liquid chromatography tandem mass spectrometry (LC-MS/MS) was performed to identify bacterial metabolites. Eight cytokines were measured using multiplexed bead-based flow cytometry.

Results: LC-MS/MS analysis revealed that all three biofilms metabolized JUUL to varying degrees, generating several end-products. JUUL also significantly altered gene expression in all three biofilms. Two-way –omics analysis revealed glycerol metabolism as a key mechanism. The mucosal transcriptome also demonstrated a significantly altered response (p<0.05, FDR) to secondary biofilms when exposed to JUUL containing 5% nicotine, with 305 genes significantly downregulated several of which encoded innate and adaptive immune responses. Cytokine analysis confirmed these findings, with a dampened pro-inflammatory response to secondary biofilms in the presence of JUUL with 5% nicotine (p<0.05, Tukey’s HSD).

Conclusions: JUUL exposure leads to a dampened pro-inflammatory response to pathogen-rich biofilms, which appears to be mediated by bacterial metabolites of JUUL aerosol. This parallels our earlier findings on the epithelial immune response to combustible tobacco products and suggests a potential mechanism for increasing risk for diseases caused by infection-mediated inflammation, notably periodontitis and oral cancer.
Altered Enamel Phenotype of ADAM10 Conditional Knockout Mice

Shifa Shahid*, Lara Rizzotto², Fatma F. Mohamed¹, R. Said¹, S. Papagerakis¹, P. Papagerakis³, B. L. Foster¹, J. D. Bartlett¹

Objectives: A Disintegrin And Metalloproteinase domain-10 (ADAM10) is a transmembrane endopeptidase that is involved in variety of biological processes due to its ability to shed membrane bound substrates. ADAM10 is essential for development of epithelial tissues as K14-Cre-ADAM10f/f deleted mice exhibit perinatal lethality because of a compromised epidermis. Our lab has previously confirmed expression of ADAM10 in the early developmental stages of the mouse enamel organ. The objective of this study was to identify the role of ADAM10 in enamel formation by generating a conditional knockout (cKO) ADAM10 mouse model.

Methods: To confirm if Cre recombinase activity was limited to ameloblasts of the enamel organ, Amelx-iCre mice were bred with Rosa26-mTmG double reporter mice. Day-5 mandibles were harvested, cryosectioned, and examined by fluorescent microscopy. Floxed Adam10 mice were bred with Amelx-iCre mice to obtain ameloblast specific Adam10 cKO mice. The apparent phenotype of these mice was documented and μCT analysis was performed on 7-8 week old cKO and wild type controls.

Results: Expression of the Amelx-iCre promoter was specific to ameloblasts as no Cre activity was detected in other enamel organ layers or surrounding tissues as determined by Rosa26-mTmG fluorescence. When compared to wild type mice, μCT images revealed that Amelx-Cre-ADAM10f/f cKO mice had a delay in initiation of enamel mineralization. The cKO mice also showed reduced enamel volume and an alteration in pigmentation of incisor enamel. Additionally, the cKO incisors did not have sharp tips suggesting that the enamel wears away easily.

Conclusions: The results showed that ADAM10 CKO mice had delayed enamel mineralization, a reduced enamel volume, altered incisor pigmentation, and lacked sharp incisor tips. These results indicate that ADAM10 plays a role in enamel development. Future experiments will more thoroughly characterize the Adam10 cKO phenotype.
Assessing Sedation Using Patient-Centered Outcomes: Behavior, Safety, Efficacy


Purpose: To evaluate pediatric dental sedation success using patient-centered outcomes for behavior, safety, and treatment.

Methods: A cross-sectional retrospective cohort study used electronic health record data of children ages 36-60 months undergoing first-time sedations for dental treatment (2015-2020) at Nationwide Children’s Hospital, Columbus, OH. Outcomes were measured: behavior success was overall-visit Frankl score 3-4; safety success was maintenance of vital signs within 2 standard deviations of age- and gender-based norms; treatment success was completion of planned dental treatment. Overall success was defined as success in 2 of the 3 measures. Descriptive statistics and bivariate comparisons compared medication regimens and administration routes.

Results: Of 824 subjects (52% female, mean age 36 months), behavior success was 49%, safety success was 84%, and treatment success was 62%. Overall success was 72%. Number of medications (excluding nitrous oxide) did not affect overall success (1 medication: 71%; 2+ medications (e.g. midazolam+hydroxyzine): 77%, \( P = .27 \)). Subjects receiving intranasal (n=543) had lower overall success rate than oral (n=189) midazolam, although the difference was not statistically significant (69% vs. 77%, respectively, \( P = .05 \)) and treatment success (63% vs. 58%, \( P = .23 \)). However, behavior success and safety success were significantly different for intranasal versus oral administration (45% vs. 60%, \( P < .001 \); and 80% vs. 92%, \( P < .001 \)).

Conclusions: Approximately \( \frac{3}{4} \) of sedations were successful. Combination regimens were neither associated with increased measure of success nor with impaired safety. All regimens exceeded 80% safety success. Intranasal midazolam proved significantly less effective for behavior and safety success than oral administration.
Functional Importance of the Bone Sialoprotein RGD Domain in Dentoalveolar Development and Alveolar Bone Healing


**Objectives:** Bone sialoprotein (BSP) is an extracellular matrix (ECM) protein present in mineralized tissues. Genetic deletion of BSP in mice causes mineralization defects in dentoalveolar development, and defective alveolar bone healing. The underlying mechanisms are not known, in part, because BSP harbors several functional domains. In vitro experiments indicate that the N-terminal RGD integrin-binding motif of BSP promotes cell signaling and adhesion. To provide new insights into the functions of BSP in vivo, we constructed a mouse model with a non-functional KAE sequence replacing the BSP-RGD domain (Ibsp^KAE/KAE). We hypothesized that inactivation of BSP-RGD would negatively affect dentoalveolar development and alveolar bone healing.

**Methods:** Dentoalveolar development in Ibsp^KAE/KAE and wild-type (WT) littermate control mice on a mixed 129/C57BL/6 genetic background was analyzed at 41-43 days-postnatal (n=5-6/group). Maxillary first molars were bilaterally extracted at 41-43 days-postnatal from Ibsp^KAE/KAE and WT mice to analyze alveolar bone repair in healing sockets at 21 days post-procedure (n=6-7/group). Tissues were analyzed using micro-computed tomography.

**Results:** In studies for the first time analyzing Ibsp^KAE/KAE dentoalveolar development on a mixed genetic background (that amplifies phenotypic severity), we found that inactivation of the BSP-RGD motif in mice did not negatively affect enamel, dentin, or alveolar bone volumes or mineral densities. Surprisingly, enamel, dentin, and alveolar bone mineral densities slightly but significantly increased in the absence of BSP-RGD. Alveolar bone healing experiments indicated no significant differences in bone volume fraction (BV/TV) or bone mineral density (BMD) in Ibsp^KAE/KAE vs. WT mice.

**Conclusions:** BSP regulates dentoalveolar mineralization in mice, though within the limits of this study, absence of the BSP-RGD domain did not cause substantial defects in tooth development or alveolar bone healing. Ongoing studies include histology of developing and healing tissues, and qPCR array to identify gene expression related to BSP-RGD.
Dental Anxiety in an Academic Setting and the Impact of Sedation

James Taylor, John Sotos, Joseph Virga, & Bryant W. Cornelius

**Purpose/Objectives:** Dental anxiety causes many patients to avoid dental treatment. Patients seeking care in an academic setting have shown greater dental anxiety compared to the general population. Sedation availability within the academic setting may reduce patient anxiety levels and improve appointment compliance.

**Methods:** The authors surveyed 118 patients of various dental clinics at The Ohio State University College of Dentistry in 2021. Participants indicated their current anxiety levels, how the availability of sedation for their dental procedure would impact their anxiety, and how sedation availability would affect their likelihood to show up for future dental appointments. Variables such as age, gender, ethnicity, and procedure severity were analyzed to determine their relationship with dental anxiety.

**Results:** The survey response rate was 78%. Participants reported a mean anxiety score of 4.01 out of 10. Participants reported this anxiety would decrease by 38% if sedation for their dental procedure were available. Females displayed the highest initial mean anxiety and greatest reduction of mean anxiety if sedation were available for their procedure. Sedation availability significantly impacted anxiety in high severity procedures.

**Conclusion:** The majority of patients in an academic setting experience moderate to severe dental anxiety. Dental anxiety, particularly in females, would decrease if sedation were to be made available for dental procedures. Sedation availability for dental patients in an academic setting may result in increased appointment compliance, reduced dental anxiety, and improved oral health.
A Comparative Study on the Effects of E-Cigarettes and Smoking on the Oral Metabolome

William Vu, Prem Preshant Chaudhary, Michelle L Scott, Purnima S Kumar

Objectives: E-cigarettes are battery operated devices that deliver nicotine and flavorings in an aerosol. The oral cavity is the first system exposed to e-cigarettes, and this ecosystem of microbes exists in homeostasis, but this balance can be disrupted by e-cigarettes, leading to a change in the byproducts of these microbes. The focus of this investigation is to discover how e-cigarettes may affect the oral metabolome of three different groups: never smokers who use e-cigarettes only, former cigarette smokers who presently use e-cigarettes, and dual users who use both cigarettes and e-cigarettes.

Methods: Saliva was collected from 55 men and women (14 never smokers, 15 dual users, and 26 former smokers). Samples were analyzed using Trapped Ion Mobility Spectrometry (timsTOF). Raw datasets were processed by using SCiLS lab 2021a software (Bruker, USA). Mass range was selected between 20-2000 m/z for assigning the regions. All m/z points were annotated by using analyte and mass spectral libraries.

Results: Analysis of 55 clinical saliva samples resulted in 3645 peaks and 503 annotated metabolites. From the collected samples, we found 606 peaks that were significant (Tukey’s HSD, p<0.05). Partial least squares-discriminant analysis (PLS-DA) analysis resulted in 3 distinct clusters that represented each of the three groups, with former smokers in between never smokers and dual users.

Conclusion: The metabolomic data shows significant clustering with little overlap between never smokers and current smokers (dual smokers). This mirrors the changes our lab previously discovered in the oral microbiomes of e-cigarette users and dual users, where dual users had a distinct microbiome separate from both e-cigarette users and smokers. The clustering of the former smokers were between that of the dual users and the never smokers, and had more overlap with the current smokers, suggesting it may require a significant amount of time before the oral metabolome can return to the same state as a never smoker.
Identification of Genes That Impact Fitness of Streptococcus mutans During Interspecies Interactions

Emily Williams, Haley Letner, Justin R. Kaspar

**Objectives:** Oral streptococci within dental plaque communities can be associated with health or disease. Previously, the caries-causing pathogen *Streptococcus mutans* has been studied primarily by itself (i.e., in mono-culture). However, *S. mutans* does not naturally exist within the oral environment alone. Whole transcriptome profiling (RNA-Seq) was used to pinpoint specific genes that were up-regulated when *S. mutans* was co-cultured with either *Streptococcus gordonii* or *Streptococcus sanguinis*. Our hypothesis is that these up-regulated genes are important for the competitive fitness of *S. mutans* when growing in the presence of health-associated oral streptococci. In this study, we compared the competitive fitness between the parental *S. mutans* strain (UA159) and mutants of our identified up-regulated genes.

**Methods:** Twelve different mutant strains, including the parental, were grown for 24 h in an initial 1:1 mixture with *S. gordonii* and *S. sanguinis*. Biofilms were then dispersed and selectively plated after serial dilution onto either kanamycin- or spectinomycin-containing agar to enumerate colony forming units (CFUs) of each species separately. A competitive index was calculated using initial and final cell counts of all strains to compare fitness levels during biofilm growth.

**Results:** We identified two out of eleven genes screened (Δ2027 and Δ2146c) that displayed a significant loss of fitness compared to the parental strain. This was due to decreases in the amount of *S. mutans* CFUs recovered, however commensal CFUs were stable across all mutant co-cultures. SMU.2027 encodes for a putative LexA-like transcriptional regulator termed *hdiR*, induced under different stress conditions. SMU.2146c contains a lytic transglycosylase-like domain that cleaves peptidoglycan.

**Conclusions:** Through our competitive fitness assay, we have identified two specific *S. mutans* genes that result in decreased fitness during co-culture as a result of their loss. This study emphasizes the importance of studying *S. mutans* in co-cultures rather than in mono-culture alone. The next step for this project is examining SMU.2027 and SMU.2146c genes more closely to determine how they assist in *S. mutans* fitness during competitive interactions. By gaining a broader understanding of co-culture competition and the genes involved, we hope to develop new therapeutic strategies to prevent dental caries in the future.
**Objectives:** Discoidin domain receptor 1 (DDR1) is a non-integrin collagen receptor that affects resident and immune cell function and matrix remodeling. DDR1 is heavily expressed at the epithelial basal layers, where it co-localizes with collagen IV, one of its major ligands. We hypothesized that DDR1 may play a role in immune-mediated diseases presenting with defects at the epithelium-connective tissue (CT) interface. The aim of this study is to assess DDR1 expression and localization in biopsies from patients with such diseases.

**Methods:** This project was approved by IRB (OSU, Columbus, OH). Histology sections of previously diagnosed cases of mucous membrane pemphigoid (MMP), pemphigus vulgaris (PV), lichen planus (LP) and fibroma were included in this study. Sections of normal gingiva were used as controls. For each disease, biopsies from 4 males and 4 females were included. DDR1 in situ hybridization (ISH) and DDR1 immunohistochemistry (IHC) were performed.

**Results:** In fibroma sections, DDR1 expression and localization was noted at the epithelial basal layer, similar to controls. Although major epithelial changes in DDR1 localization for MMP and PV sections were not observed, round cells with strong peripheral DDR1 staining were found in the CT, immediately below the basement membrane. For LP, and especially for cases with heavy CT inflammatory infiltrate, DDR1 expression was decreased at the epithelial basal layer, but was present in the inflammatory infiltrate. IHC showed DDR1-stained cells in the CT inflammatory infiltrate of LP, similar to MMP and PV. Further experiments are underway to determine the identity of these cells.

**Conclusions:** DDR1-stained round cells, consistent with immune cells, were present in the CT of MMP, PV and LP cases, close to the basement membrane. This suggests a potential role for DDR1 in auto-immune processes implicated in diseases resulting in defects at the epithelium-CT interface.
Natalie Andras - 01
**DDS/PhD Candidate**
Dentoalveolar Defects from Neural Crest Conditional Deletion of Bone Sialoprotein

Chandler Ashman - 02
**Neuroscience Student**
Tgfr2 in dental pulp cells guides sensory innervation in developing teeth

Madisen Bangs - 03
**Neuroscience Student**
Streptococcus mutans Isolates Exhibit Diverse Phenotypes Dependent on Commensal Competitor

Jordan Batagower - 04
**Biology Student**
Development of Fluorescent Microscopy Protocol for Analysis of Multi-species Oral Biofilms

Paige Bending - 05
**Neuroscience Student**
Growth Environment and its Impact on Competition Between Oral Streptococci

Robert Bettinger - 06
**Microbiology Student**
Identification of Genes that Impact Fitness in Streptococcus gordonii

Michelle Blyumin - 07
**Biology Student**
Rat tooth extraction protocol for proteomics analysis of the dentin matrisome

Adam Bouchendouka - 08
**Biomedical Science Student**
Impact of Growth Environment on Quorum Sensing Inhibition in Streptococcus mutans

Timothy Dang - 09
**Human Nutrition Student**
Isolates of Caries Pathogen Streptococcus sobrinus Display Diverse Phenotypes

Emma Dixon - 10
**Dental Student, Year 1**
A narrative review on risk assessment tools as prognostics of implant supported dental restorations

Kelly Doan - 11
**Dental Student, Year 4**
Alveolar Bone Mineral Density in Women With Aging

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Growth Environment Impacts Interspecies Interactions Between Oral Streptococci

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