2023 Research Day
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: Susan R. Mallery, DDS, MS, PhD
Professor & Chair, Division of Oral and Maxillofacial Pathology
Title: Oral Cancer Chemoprevention by Local Delivery
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: Nisha D'Silva, BDS, MSD, PhD
Donald Kerr Endowed Collegiate Professor, University of Michigan
School of Dentistry, Professor of Pathology at the University of Michigan
Medical School and University of Michigan Rogel Cancer Center
Title: Oral Cancer Progression: Hitting a Nerve
9:00–10:00 a.m.

1.5 CDE credits (all speakers) available for licensed practitioners

POSTER PRESENTATIONS

Poster Display Location
Building B Atrium and adjacent hallway
10:00–11:30 a.m.

AWARDS PRESENTATION

Dean's Awards for Excellence in Research
Building A, Lecture Room 1160
Scott R. Schricker, PhD, Associate Professor, Director of Student Research
John D. Bartlett, PhD, Professor & Associate Dean for Research
4:30–5:15 p.m.
ORGANIZING COMMITTEE

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 and Support
Sherri Doughty
Tina Adathakkar
William Johnston, PhD
Michelle Layana
Andrew Peters
Emma Frey
Don Gray

Marketing & Communications
Bethany Waal

OHIO STATE DENTISTRY STUDENT RESEARCH GROUP

President
Kazune Pax (DDS/PhD year 6)

Vice President
Michelle Scott (DDS/PhD year 6)

Secretary
Natalie Andras (DDS/PhD year 4)

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Shifa Shahid (PhD student)

Advocacy Rep
Adam Wade (D1)

Historian
Aakriti Chaudhry (D3)
I am pleased to have this opportunity to welcome you to Research Day!

We host this event each year so our college community and guests can learn about the scientific achievements made by our faculty and students. We have a thriving research program in biomaterials, bone and muscle biology, the microbiome, psychoneuroimmunology, oral cancer treatments using natural agents, and more. It is a point of pride that our collective efforts are helping to expand the knowledge base of our profession. In addition to presenting our researchers’ accomplishments and innovations, Research Day showcases the ground-breaking discoveries made by some of the world’s most respected researchers in dentistry and fields related to oral health.

This year’s distinguished lecturer, Nisha D’Silva, BDS, MSD, PhD, is the Donald Kerr Endowed Collegiate Professor at the University of Michigan School of Dentistry. She also serves as a Professor of Pathology at the University of Michigan Medical Center and is a Research Member at the University of Michigan Rogel Comprehensive Cancer Center. As an educator, pathologist, and cancer biologist, Dr. D’Silva’s research on head and neck cancer focuses on the mechanisms of tumor progression and treatment resistance. Her work has been funded by the National Institutes of Health - National Institute of Dental and Craniofacial Research and the National Cancer Institute. We welcome Dr. D’Silva as our distinguished lecturer, and we look forward to her presentation, “Oral Cancer Progression: Hitting a Nerve.”

Our faculty speaker for this year’s event is Susan Mallery, DDS, MS, PhD. An Ohio State alumna and Chair of the Division of Oral and Maxillofacial Pathology, Dr. Mallery is a nationally recognized researcher whose work is focused on the chemoprevention of oral cancer by natural products. Her presentation is titled “Oral Cancer Chemoprevention by Local Delivery.”

This year’s Research Day includes oral and poster presentations by our dental students, post-doctoral fellows, and residents. To recognize those whose work is judged as outstanding, we will present awards at a reception that will be accessible via Zoom.

Please join me in thanking Dr. John Bartlett, Associate Dean for Research, and the Office of Research staff members who have prepared this exceptional event that is an enriching experience for all of us!

Carroll Ann Trotman, BDS, MA, MS
Professor and Dean
As the Associate Dean for Research, it is my pleasure to welcome you to the College of Dentistry’s 39th 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 three DDS students, top three graduate students/residents, and for the top undergraduate student, hygiene student, and postdoctoral fellow.

Research Day prepares our students and postdoctoral fellows for national and international competitions, including several sponsored by the American Association of Dental, Oral and Craniofacial Research (AADOCR) or International Association for Dental Research (IADR). 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 Dentsply Sirona and the AADOCR. 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, Susan R. Mallery, DDS, PhD, is our Research Day faculty presenter. She is Professor and Chair of the Division of Oral and Maxillofacial Pathology. Dr. Mallery has invented an oral patch that can deliver therapeutics to the inside of the mouth. She is actively involved in research focused on the discovery of viable treatments and treatment systems to ameliorate and/or cure oral cancer. And, she has performed several promising clinical trials demonstrating regression of precancerous oral lesions. Dr. Susan Mallery has discovered, patented and formed companies, such as Sirona Therapeutics, based on her novel oral cancer treatments that directly treat the affected site. She is a well-funded NIH investigator who collaborates with both universities and corporations to achieve her cancer research goals. Additionally, she was inducted into the Ohio State University Athletic Hall of Fame for her exploits in track and field. She is therefore a well-rounded individual with an overarching focus on health.

We are pleased to welcome Nisha J. D’Silva, BDS, MSD, PhD, as our Distinguished Lecturer. Dr. D’Silva earned her dental degree from the University of Bombay, India and earned her doctoral degree in Oral Biology from the University of Washington School of Dentistry. She is the Donald Kerr Endowed Collegiate Professor at the University of Michigan, School of Dentistry. She is also a Professor in the Department of Pathology at the University of Michigan Medical School and is a member of the University of Michigan Rogel Cancer Center. Dr. D’Silva studies translational research in head and neck cancer focusing on biomarkers and molecular mechanisms of tumor progression and treatment resistance. She is currently funded by a NIH/NIDCR R35 Sustaining Outstanding Achievement in Research Award and she is also funded by the National Cancer Institute. She has published her research in outstanding journals such as Cancer Research, Nature Communications, and Cell. Dr. D’Silva has earned a variety of awards for her outstanding research pursuits. These include the “Distinguished Scientist Award for Oral Medicine and Pathology Research” from the International Association of Dental Research, “the Rod Cawson Prize” from the International Association of Oral Pathologists and the Royal College of Physicians.

continued
and Surgeons of Glasgow, and the “Irwin Mandel Distinguished Mentoring Award” from the AADOCR. We are thrilled to have Dr. D’Silva as our Distinguished Lecturer for our 39th Research Day. Her presentation is titled, “Oral Cancer Progression: Hitting a Nerve”.

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 OSU College of Dentistry Research Committee, I would like to welcome you to Research Day 2023. I want to start by thanking Dr. Bartlett and the Office of Research at the College of Dentistry for their amazing work on keeping the tradition alive through virtual research day presentations for the last two years. But this year is different. We are so excited to be back to an in-person Research Day. This is a first for most of our current dental students.

Research Day has been a key component of our dental curriculum since the beginning. It is a day that allows the entire college community to get together to celebrate our students’ achievements in research and to learn about some of the inspiring work happening behind the doors within the Postle Hall. We share the excitement and enthusiasm of clinical and bench type research conducted within our college with the help of dedicated mentors and supportive administration.

Once more, a great scientific program has been prepared for this year’s Research Day. Our distinguished speaker Dr. Nisha D’Silva is joining us from Michigan. She has significant published work on Cancer Neurosciences. She will be covering the topic of “the role of the nerves on oral cancer progression”. We will also have a chance to listen to our own Dr. Susan Mallery and catch up with her recent achievements in oral cancer research. Of course, our students are spending significant time to put their presentations together and get ready for this event. We cannot wait to share all of these with all of you.

We are so happy that we will be meeting in-person this year. This will allows us to mingle with each other and catch up with rich discussion opportunities between presentations. Please join me in supporting our young researchers and to enjoy a day full of new data coming out of our college’s laboratories and clinics.

Binnaz Leblebicioglu DDS, MS, PhD
On behalf of Student Research Group (SRG), I am excited to welcome you to the annual Research Day! We are so excited to be back in-person this year! Be sure to take advantage and walk through all the posters to see everyone's hard work over the past year.

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. 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, Nisha D'Silva, BDS, MSD, PhD, will discuss the role of nerves in both the progression and response of oral cancers. Dr. D'Silva is a Donald Kerr Endowed Collegiate Professor at the University of Michigan School of Dentistry. She also holds an appointment with the Medical School. Her research focuses on head and neck cancers, specifically on tumor-environment interactions that promote growth, spread, and resistance. I have many friends in Dr. D'Silva's lab and they all love working with her. She is a genuinely nice 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 beloved Susan Mallery, DDS, MS, PhD. While we all know her for her passion in teaching us clinical oral pathology, she also spends her time researching chemoprevention of oral cancers using natural products. If you've never stopped to chat with her in the hall, be sure to take a moment to get to know the other side of Dr. Mallery.

Furthermore, the presentations given today help prepare the students for national research competitions at the American Association for Dental and Craniofacial Research (AADOCR), the ADA/Dentsply Student Clinician Program, and the Hinman Student Dental Research Symposium. Students receive valuable feedback from senior researchers, distinguished faculty, and fellow peers. We applaud all the participants for their tremendous effort to complete their research and present their work. Students recognized for their outstanding achievements at will be honored at the award ceremony and reception 4:30 today.

Finally, please join me in extending our sincerest gratitude to our Associate Dean for Research, Dr. John Bartlett, the Office of Research staff, and SRG members who help make this event possible. Everyone dedicated a lot of time and effort to prepare an extraordinary program and provide us all with a meaningful and inspiring experience. We hope you appreciate the in-person event as much as we do!

Kazune Pax, SRG President
Susan R. Mallery, DDS, PhD, is a tenured professor at The Ohio State University, College of Dentistry and she is chair of the Division of Oral and Maxillofacial Pathology. Dr. Mallery’s research focuses on identification and local delivery of oral cancer chemopreventives. Accordingly, her research extends from bench top discoveries, to animal models and finally to human clinical trials. Because the oral cavity is visually accessible, it is an ideal site for local delivery formulations. Dr. Mallery’s strategy, which entails controlled release of chemopreventive agents at the premalignant site, ensures therapeutically relevant levels are achieved at the treatment site without drug-related systemic effects.

In conjunction with Ohio State’s Drug Development Institute and Ohio State’s Office of Technology Licensing, Dr. Mallery served as the Principal Investigator for the Co-Development project between the Columbus-based pharmaceutical company Venture Therapeutics and Ohio State. Ohio State collaborated with the University of Michigan in this co-development effort. This partnership enabled creation of Sirona Therapeutics, which is the first co-development project and development company initiated by Ohio State. Dr. Mallery has also been very successful in obtaining NIH funding to support her cancer chemoprevention studies. She has had NIH funding since 1985. In 2019, Dr. Mallery was awarded a five-year NIH/NCI grant (R01 CA227273). This is large R01 grant (total costs $3,491,528) titled, “Assessment of Chemopreventive Effects of a Mucoadhesive Fenretinide Patch on Premalignant Oral Epithelial Lesions,” funds a Phase Ib clinical trial to assess chemopreventive efficacy of the fenretinide patch on premalignant oral epithelial lesions. In December of 2021, Dr. Mallery was awarded another large five-year NIH/NCI grant which will fund a Phase 0 clinical trial to assess a nanoparticle releasing oral thin film for oral squamous cell carcinoma chemoprevention. This nanoparticle technology, designed to provide chemopreventive field coverage throughout the mouth and complements the lesional site-directed fenretinide patch. Both the fenretinide patch and the Janus nanoparticle technologies were awarded US patents.

Therefore, Dr. Mallery has made great strides toward her goal of oral cancer chemoprevention and we are excited to learn about Dr. Mallery’s research on cancer chemoprevention during her Research Day presentation titled, “Oral Cancer Chemoprevention by Local Delivery.”
DISTINGUISHED LECTURER
DR. NISHA D’SILVA

Nisha D’Silva, BDS, MSD, PhD, is the Donald Kerr Endowed Collegiate Professor at the University of Michigan School of Dentistry (UMSD). She is also professor of pathology at the University of Michigan Medical School and a member of the University of Michigan Rogel Cancer Center. Dr. D’Silva is a dentist-scientist who earned her dental degree from the University of Bombay and her doctorate from the University of Washington in Seattle. Dr. D’Silva performs translational research in head and neck cancer with a focus on biomarkers and molecular mechanisms of tumor progression and treatment resistance. She has over 100 peer-reviewed publications including publications in prestigious journals such as Cancer Research, Journal of Experimental Medicine, Nature Communications, and Cell. Dr. D’Silva is in demand as an invited speaker. In 2022, she gave ten different talks including at Indiana University, University of Rochester, Travis Air Force Base in California, and at Harvard University. Dr. D’Silva currently has an NIH/NIDCR R35 Sustaining Outstanding Achievement in Research (SOAR) Award titled, “Improving Survival in Oral Cancer by Disruption of Tumor Progression”. She is currently a principle investigator, co-principle investigator, or a co-investigator on seven additional grant awards. Since the year 2000, Dr. D’Silva has had continuous NIH funding for her compelling research projects.

Dr. D’Silva’s honors and awards include: The Rod Cawson Prize Lecture awarded by the International Association of Oral Pathologists and the Royal College of Physicians and Surgeons of Glasgow, the Distinguished Scientist Award in Oral Medicine and Pathology Research from the International Association of Dental Research, and the Irwin Mandel Distinguished Mentoring Award from the American Association of Dental Research. She has also earned the Michigan Institute for Clinical and Health Research Distinguished Mentor award and, she was selected as a Rogel Scholar for the University of Michigan Rogel Comprehensive Cancer Center. Dr. D’Silva is also a Fellow of the American Association of Dental Research and is a Diplomate of the American Board of Oral and Maxillofacial Pathology. We are thrilled to have the accomplished Dr. D’Silva as our Distinguished Lecturer for our 39th Research Day. The title of her talk is, “Oral Cancer Progression: Hitting a Nerve.”
Committees and Judges

OUR SINCERE APPRECIATION TO THE FOLLOWING INDIVIDUALS

Student Research Awards Committee
Dr. Homa Amini '24
Mr. Don Gray, Ex-officio
Dr. Yuan-Lynn Hsieh '24
Dr. Justin Kaspar '24
Dr. Ashok Kumar '25
Dr. Beau Meyer '24
Dr. Kazune Pax '23, Graduate student
Dr. Elisandra Reyes-Perez '24
Dr. Scott Schricker, Chair, Ex-officio
Dr. Anjum Shah '24

Research Committee
Dr. Ehsan Azadani '23
Dr. John Bartlett, Ex-officio
Dr. Clifford Beall '24
Dr. Melissa Drum '24
Dr. Hany Emam '23
Dr. Justin Kaspar '24
Dr. Do-Gyoon Kim '24
Dr. Binnaz Leblebicioglu, Chair '24
Dr. Kazune Pax '23, graduate student
Dr. Sarah Peters '24
Dr. Peter Reiser '24
Dr. Scott Schricker, Ex-officio, non-voting

AND TO OUR DISTINGUISHED JUDGES FOR GRACIOUSLY VOLUNTEERING THEIR TIME AND EXPERTISE FOR THIS EVENT

Dr. Clifford Beall
Ms. Beth Chartier
Dr. Lewis Claman
Dr. Jim Cray
Dr. Melissa Drum
Dr. Brian Foster
Dr. Sara Fowler
Ms. Rebecca Henderson
Dr. John Kalmar
Dr. Justin Kaspar
Ms. Rachel Kearney
Dr. Do-Gyoon Kim
Dr. Thomas Knobloch
Dr. Beth Lee
Dr. Diana Leyva del Rio
Dr. Luiz Meirelles
Dr. Beau Meyer
Dr. Fatma Mohamed
Dr. Leo Nassani
Dr. Sarah Peters
Dr. Peter Reiser
Dr. Scott Schricker
Dr. Robert Seghi
Dr. Shilpa Shah
Dr. John Sheridan
Dr. Dimitris Tatakis
Dr. Janice Townsend
Dr. Gabriela Weiss

Special thanks goes to William Johnston, PhD, and Michelle Layana for statistical assistance.
Fellowships, Awards, and Recognitions

The College of Dentistry’s 38th Annual Research Day, held on February 25, 2022, featured undergraduate, dental and dental hygiene students, predoctoral 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 judges and other interested individuals.

Included in the awards for the 2022 Student Research Competition was the SCADA Award cosponsored by Dentsply Sirona and the American Association for Dental, Oral and Craniofacial Research (AADOCR). The winner of this award qualified for entry into the national Student Competition for Advancing Dental Research and its Application at the annual AADOCR General Session. The 2022 SCADA Award winner was Natalie Andras.

The second-place award for the Student Research Competition is the Alumni Research Merit Award. The winner of this 2022 award qualified for entry into the Hinman Student Research Symposium competition, with support from the Thomas P. Hinman Dental Society, the AADOCR, and Procter & Gamble. The 2022 Alumni Research Merit Award winner was Joe Kainrad.

The third-place award for student researchers is the Alumni Research Achievement Award. The winner is qualified to enter the Hinman Student Research Symposium competition. The 2022 Alumni Research Achievement Award winner was Parker Heiner.

The following recipients were chosen as winners of the 2022 Dean’s Award for Excellence in Research for their innovative projects. William Vu, Joe Kainrad, and Parker Heiner won the first-, second-, and third-place Dental Student Awards. Kelly Doan won the AADOCR Research Day Award. Natalie Andras and Shifa Shahid tied for first place and Michelle Scott was awarded second place for the Graduate Student Awards. Fatma F. Mohamed received the Postdoctoral Fellow Award; and William Vu won the award for the CCTS Best Clinical and Translational Abstract.*

*This award is sponsored by The Ohio State University Center for Clinical and Translational Science.
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Induced periodontitis in 3xTG mice increases inflammation and bone loss

Daniela Jimenez-Harrison, Rami Alsabbagh, Michael Butler, Ashton Taylor, Menaz Bettes, Ruth Barrientos, Sarah B. Peters

**Background:** Alzheimer’s disease (AD) is the most common form of dementia in aging adults. Studies suggest that inflammation plays a key role in AD progression but little is known about the signaling pathways driving this. Periodontal disease (PD) is an aging disease in which chronic inflammation leads to bone and tooth loss in 20-50% of the global population. Several studies have found a positive correlation between AD and PD, but the mechanisms underlying their relationship remain unclear. We hypothesized that inflammation associated with periodontitis could exacerbate AD progression and that the AD predisposition may similarly exacerbate periodontitis symptoms.

**Approach:** We induced periodontitis in transgenic AD (3xTg-AD) and WT mice with a silk ligature tied around the right second maxillary molar. We assessed bone loss with *in vivo* microcomputed tomography on days 0, 7 and 14. Levels of hippocampal inflammatory cytokines and neuronal receptors were measured using RT-PCR.

**Results:** WT and AD mice showed no differences in bone loss at 14 days. The non-ligature side showed minimal bone loss in both genotypes. qPCR data indicated an increase in inflammatory cytokine gene expression in the hippocampus of AD mice as compared to WT mice. There was also a significant decrease in gene expression of glutamate receptors and synaptic elements in the AD hippocampus after ligature placement.

**Conclusion:** PD-evoked bone loss in AD mice led to exaggerated hippocampal neuroinflammation and synaptic dysregulation. Studies are currently underway to assess behavioral modifications in AD mice with induced PD to provide a thorough characterization of how oral inflammation may lead to neuronal degradation. Future studies are planned to determine the mechanism that links PD with AD and other neurodegenerative diseases.
Gene-Editing Provides Functional Evidence for the Ectomesenchymal Origin of Cementoblasts

Natalie L. Andras1, Michael B. Chavez1,2, Fatma F. Mohamed1, Michelle H. Tan1, Tamara N. Kolli1, Alexis N. Powers1, Brian L. Foster1

Objectives: Hypotheses regarding the origin of cementoblasts are mired with controversy, which hinders cementum regeneration efforts. The “classical-hypothesis” postulates cementoblasts arise from ectomesenchyme, whereas the “alternative-hypothesis” states Hertwig’s epithelial root sheath undergoes an epithelial-mesenchymal transformation. To delineate the origin of cementoblasts, we propose a novel, functional approach whereby bone sialoprotein (BSP), a protein essential for cementum formation and selective for cementoblasts, is conditionally ablated from ectomesenchymal or epithelial tissues. We hypothesized cementoblasts arise from ectomesenchyme.

Methods: We developed a mouse carrying a floxed Ibsp allele (Ibspfl/fl) and conditionally deleted Ibsp from ectomesenchymal (Wnt1-Cre2) and epithelial (K14-Cre) tissues. Mandibles were harvested from wild-type control (WT;Ibspfl/fl) and conditional-knockout (cKO;Wnt1-Cre2+;Ibspfl/fl and K14-Cre;Ibspfl/fl) mice (n=6/group/timepoint) at 30- and 90-days-post-natal. Dentoalveolar development was analyzed by micro-computed tomography, histology, immunohistochemistry, and in-situ hybridization.

Results: BSP protein was present in WT and BspK14-cKO cementum but absent in BspWnt-cKOs. In BspWnt-cKOs, cementoblast Ibsp mRNA expression was reduced, but deposition of osteopontin, another cementoblast marker, was unaffected. Compared to WT, the volumes, densities, and histological organization of dentoalveolar tissues in BspK14-cKOs were unchanged. Developmental analyses of BspWnt-cK0 first molar roots revealed reduced acellular (≥80%;p<0.0001-0.001) and cellular (24-40%;p<0.01-0.05) cementum, increased cellular cementum cementoid, and patchy acellular cementum. Cementum defects were not associated with changes in serum alkaline phosphatase, calcium, or phosphate. Secondary, structural defects along the cementum-PDL-alveolar bone (AB) interface arose in BspWnt-cKOs, including excessive AB osteoid, decreased volume (40-60%;p<0.0001) and mineral density (4-6%;p<0.0001-0.001) of AB proper, increased PDL volume (28-49%;p<0.001-0.0001), detachment and disorganization of PDL fibers, epithelial downgrowth, extensive root and AB resorption, and increased osteoclast number.

Conclusions: Ectomesenchymal, not epithelial, BSP ablation resulted in impaired cementum formation, which led to periodontal breakdown and dysfunction. These results provide the first, functional evidence for the ectomesenchymal origin of cementoblasts and will aid in developing targeted therapies for cementum regeneration.

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Streptococcus mutans Isolates Exhibit Diverse Phenotypes Dependent on Commensal Competitor

Madisen Bangs, Paige Bending, Delaney Hancock and Justin R. Kaspar

**Objectives:** *Streptococcus mutans* (SMU), a common dental caries pathogen, exhibits both antagonistic and synergistic relationships with other commensal oral streptococci strains. Our group has a collection of SMU isolates from diverse locations around the globe that have previously been shown to display different growth phenotypes. However, there is minimal previous exploration of behavior of these isolates during interspecies interactions. 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:** A selection of 12 oral species were cocultured with 8 selected SMU isolates. We compared the accumulated biomass by the crystal violet biomass assay and also evaluated biofilm formation/structure via widefield fluorescent microscopy. Resulting data was plotted and analyzed via GraphPad Prism.

**Results:** We found no common biomass trends applicable to all coculture interactions, indicating different levels of interspecies cooperation amongst the isolate collection. For instance, cocultures with disease-associated *Streptococcus sobrinus* displayed a diverse array of phenotypes. SMU_089 and 098 each exhibited robust monoculture biofilm formation, but decreased coculture biomass in the presence of S. sobrinus. However, SMU_104 had low monoculture biomass, and biofilm formation was increased in the presence of S. sobrinus. Fluorescent microscopy data revealed differing levels of biomass between groups within our isolate panel.

**Conclusions:** It is evident that not all isolates behave the same way in the presence of different competing species. This investigation reinforces the diversity of S. mutans isolate behaviors and highlights the importance of studying diverse isolates rather than only a singular reference strain for the species. Moving forward, we will quantify the amount of demineralization on hydroxyapatite disks over a 24-hour period and perform advanced imaging of the disk surface. By studying interspecies interactions, we can gain better understanding of critical factors that drive the fitness of pathogens such as *S. mutans*.
Characterization of Oral Interspecies Interactions via RNA Sequencing and Microscopy

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. Here, we aimed to observe and quantify streptococcal species interactions between *Streptococcus mutans* and oral streptococci commensals within cocultured biofilms.

Methods: We performed RNA-seq on monocultures of *S. mutans* and cocultures of *S. mutans* with oral streptococci commensals. We bioinformatically analyzed and quantified the data to determine differentially expressed genes between mono- and cocultured conditions. For microscopy, we utilized a *S. mutans* strain that constitutively expressed green fluorescent protein (**gfp**) and commensal streptococci that constitutively expressed *dsRed-ExpressII*. After 24 h of growth, biofilms images were captured with a Biotek Lionheart FX microscope and the Biotek Gen5 software was used to quantify specific features of each individual fluorescent channel, such as number of microcolonies and biomass.

Results: From our RNA-seq data sets, we observed 231 differentially expressed genes between our coculture conditions compared to the monoculture. Of the 231 genes, 26 were differentially expressed in all 4 datasets. During microscopy, 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.

Conclusions: During RNA-seq and fluorescent microscopy we were able to successfully visualize and quantify differential gene expression and biomass changes between cocultures of oral streptococcus species. These techniques aid our ability to further identify a possible causal explanation for the way the bacterial interactions occur. Through the success of these, we expect to gain more insight into what may be occurring in vivo during biofilm formation of streptococci in the oral cavity.
SSRI Drug Exposure in utero Affects Development of the Palate in a Murine Model

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Objectives: Antidepressants, specifically Selective Serotonin Re-uptake Inhibitors (SSRIs), that alter serotonin metabolism are currently the most prescribed drugs for the treatment of depression. There is some evidence to suggest these drugs contribute to birth defects, specifically craniofacial malformations. As jaw development is often altered in craniofacial birth defects, the most common birth defects seen clinically, the purpose of this study was to interrogate the effects of in utero SSRI exposure in a preclinical model of maxillary, palatal, and dental development. We hypothesized that in utero exposure to SSRI would affect dimensions of both the palate and maxillary molars of murine mice models.

Methods: To test this hypothesis, wild type C57BL6 mice were used to produce litters that were exposed in utero to an SSRI, Citalopram (500 Mg/day). Murine skulls from P15 pups were subjected to MCT and images were obtained using a SkyScan 1176 (Bruker Kartuizerseg 3B, 2550 Kontich, Belgium) scanner. Scans were analyzed using AnalyzePro for change in shape and composition of the facial complex using cephalometric analyses.

Results: Results suggest SSRI exposure resulted in offspring having significantly shorter (p=0.017) and narrower palates (p=0.013) using multiple measures compared to control untreated pups. Upon examination of dentition there was minimal impact on molar length and width (p>0.05).

Conclusions: These resulting craniofacial morphologies have been shown to increase risk of malocclusion, sleep apnea and difficulties nursing in humans. The results of this study reveal SSRI exposure may interrupt maxillary and more specifically palatal growth in a model of development giving insight into the expectation that children exposed to SSRIs may require orthodontic intervention.

KEYWORDS: SSRI, Serotonin, Maxilla, Palate, Molar.

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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 coculture growth with *S. mutans* via an RNA-Seq experiment. We hypothesize that differentially expressed genes within this dataset, particularly upregulated 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 upregulated from the previous coculture RNA-Seq data. Selected genes underwent allelic replacement with an erythromycin antibiotic marker to construct individual mutant strains. Growth of the new mutant strains were assessed in different medium conditions. Crystal violet biomass assays were used to compare biofilm formation both in monocultures and in cocultures with *S. mutans*. The fitness of each mutant was analyzed through competitive index assays 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 competitive index assays, it was determined that Δ1892 had a significant change in growth in coculture with *S. mutans* supplemented with glucose and N-acetylglucosamine.

**Conclusions:** A gene in *S. gordonii* potentially involved in interspecies interaction with *S. mutans* was analyzed. Further research will continue characterizing the role of SGO_1892 in interactions with *S. mutans* by analyzing growth in various carbohydrates. 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.
Analysis of the dentin matrisome composition over time

Michelle Blyumin, Sarah B. Peters, PhD

Objective: Neurite outgrowth increases as part of a robust repair process in young teeth. This is due in part to bioactive proteins in dentin (BPiD) including neurotrophins. Typically, the BPiD are sequestered in tissue within the dentin extracellular matrix (ECM) and preserved through the formation of proteoglycan bonds during dentinogenesis. They are released only in response to injury or infection. Neurite outgrowth is decreased in aged rats with dentin injury, which suggests that both the tooth structure and the dentin growth factors present have an effect on tooth maintenance. However, the impact of aging on these tissues and the degree to which dental pulp tissue can repair dentin remains unknown. These BPiD possess tremendous potential for translation into regenerative dental therapeutics.

Methods: First, second, and third rat molars were extracted from maxillae and mandibles from young (3 months old; n=4) and old (24 months old; n=4) male samples. Crown and root portions were separated from one another and pulp was removed. The mineralized tissue was cryogenically milled for subsequent protein extraction followed by proteomics analysis and statistical analysis.

Results: Proteomics analysis demonstrated that crown and root BPiD were statistically different and changed with age. We classified the BPiD into six categories including: secreted factors, ECM-affiliated proteins, ECM regulators, proteoglycans, ECM glycoproteins, and collagens. Additionally, protein identities and fold changes were specified. When comparing protein composition between roots and crowns, we found proteins were upregulated in roots. This was consistent in comparisons for young and old rats. When young roots were compared to old roots, proteins were observed to be upregulated in old roots. These findings were consistent for crowns.

Conclusion: Our results demonstrate age-related changes in both crowns and roots of male rat molars and serve as a foundation to begin designing precision-based regenerative treatments for vital pulp therapies.
Dental Integration in Pediatric Primary Care: A Mixed Method Study

Natalie Bussard, Paul Casamassimo, Beau Meyer, Andrew Wapner, Jin Peng, Homa Amini, Susan Lawson; Charitha Gowda

Purpose: Medical and dental collaboration promotes patient-centered health care. This study compared age of first dental visit and dental outcomes between children who did and did not receive preventive oral health services (POHS) in pediatric primary care settings. Attitudes, beliefs, and best practices within pediatric primary medical care were assessed relating to oral health integration for young children.

Methods: This two-part mixed-method study analyzed dental claims of a subset of Medicaid-enrolled children age 1-5 years in southern Ohio from 2017 to 2021. Descriptive statistics compared the age of first dental visit between children who did and did not receive POHS in medical settings, represented by physician-applied fluoride varnish. Subsequent dental utilization outcomes were compared including preventive visits, treatment in dental clinic, treatment under general anesthesia, and dental treatment in the emergency department. Semi-structured interviews with Ohio pediatricians were conducted to better understand their attitudes, beliefs, and best practices related to POHS and dental team integration in the primary care setting. Interview transcripts were analyzed thematically.

Results: Children that received POHS in medical settings (n=8,951/33,293) had their first dental visit at a significantly earlier age (3.5 years) compared to children who did not receive these services (4.4 years, P<.001). The idea of integrating a dental provider within pediatric primary care clinics was deemed highly desirable by pediatricians. However, attitudes were mixed regarding ability to integrate dental care within primary care, with key barriers including time constraints, reimbursement concerns, and willingness of personnel to adapt to new processes.

Conclusion: Preventive oral health services within the pediatric medical home is associated with earlier age of first dental visit and may improve oral health outcomes for children. Active collaboration between primary care and dental providers is critical to successfully implement oral health integration in primary care settings.
Antibiotic resistance acquisition in a de-novo community versus established community

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Abstract: Objective: Periodontists sometimes prescribe antibiotics to help control periodontitis, however, it is unknown how the antibiotics affect the re-colonization of a subgingival pocket. The objective of this study was to determine the effects of antibiotics in the colonization of the oral cavity in a longitudinal study design.

Methods: Plaque samples were collected using paper-points from 9 systemically healthy Caucasian women from 2 sites, tooth #12 and #22, where #12 was not cleaned and #22 was cleaned before antibiotics. Samples were collected at five time points: baseline initial visit, 1 week, 3 weeks, 6 weeks, and 12 weeks post a course of Amoxicillin 875 mg bid for 7 days. RNA was isolated using MagMAX Total Nucleic Acid Isolation Kit and rRNA deletion and bacterial mRNA purification done using MICROBExpress Kit. Whole genome shot-gun sequencing was performed on Illumina NovaSeq platform. Sequences were assembled using MEGAHIT. Taxonomy was assigned using Kraken against the Human Oral Microbiome Database (HOMD). Sequences were functionally annotated against the KEGG, CARD, and VFDB databases. LEfSe was used to find differentially expressed genes.

Results: The alpha and beta diversity of both sites within the mouth were not significantly different prior to the administration of antibiotics (Shannon p-value=0.401, Kruskal-Wallis; Bray-curtis p-value=0.222, PERMANOVA). Virulence factors were acquired early in de-novo communities, within 1st week of antibiotic administration (p-value<0.05, log LDA>2, Kruskal-Wallis) with relative up-regulation of gene expression of biopolymer transport proteins, outer membrane protein, vancomycin resistance, and multivalent adhesion molecules. Established microbial communities experienced minimal changes in relative gene expressions for up-to 3 weeks after antibiotics, with majority of upregulation in gene expression experienced between 3 to 6 weeks post-antibiotics course (p-value<0.05, log LDA>2, Kruskal-Wallis).

Conclusion: Virulence factors are acquired early, within 1 week of antibiotic administration in a de-novo community colonization. However, it can take up to 3 to 6 weeks for antibiotic resistance to be established in an established microbial community.
Effects of a High Phosphate Diet on Bone Health

Andrew Cheline, Peter Tsatalis, Irene Huh, Momoko Karashima, Kristin Nguyen, Minji Kim, Cynthia Murray, Daniel A. Branch, Kedryn K. Baskin PhD, Beth S. Lee PhD, Do-Gyoon Kim PhD

Objectives: Increased intake of processed foods leads to elevated serum inorganic phosphate (PI) levels, which may lead to chronic kidney disease, adverse cardiovascular complications, and skeletal muscle dysfunction. However, the effects of inorganic phosphate on bone health, particularly of alveolar and mandibular bone, are not fully understood. Therefore, the aim of this study was to determine whether a high phosphate diet could alter site-specific mineralization of bone by comparing the jaw and femur in mice.

Method: 20 C57BL6N mice (20- to 24-week-old males) were fed a normal phosphate (NP; n=10) or a high phosphate (HP; n=10) diet with 0.9% and 2.3% total PI over the course of 12 weeks, respectively. A mandible and a femur were randomly dissected from each animal and scanned using a micro-computed tomography at 20-micron voxel size. A whole bone (WB) of mandible was determined after digitally removing teeth and alveolar bone (AB) isolation within 200 microns away from the tooth surface. The cortical bone (CB) and trabecular bone (TB) of the femur were separated from the WB of the femur. A CT attenuation value of each bone voxel, which is proportional to tissue mineral density (TMD), was calibrated. A histogram for TMD was created to compute mean, lower and upper 5th percentile (Low and High) values. A t-test was performed to compare the TMD parameters between the two groups.

Results: The WB and AB of mandible and the WB and CB of femur had significantly lower TMD Mean, Low and High, values of the HP group than those of the NP group (p≤0.05).

Conclusion: These findings indicate that a high PI diet likely increases the risk of tooth loss and bone fracture by reducing mineral density of jaw AB and femur CB, respectively.
Effects of a High Phosphate Diet on Bone Health

Jeffrey Chen, Damian Lee, Dimitris Tatakis, Binnaz Leblebicioglu

Objective: Pre-implant augmentation/corrective procedures are routinely performed to overcome limited ridge volume especially in esthetic zone. We previously conducted a retrospective study and determined that mean number of pre-implant repeated surgeries was $3.14 \pm 1$ (ranging 1 to 7) to reach acceptable bone volume. The aim of this current prospective study is to incorporate patient based into clinical outcomes specifically focusing on esthetic zone.

Material and Methods: E-chart system is used to locate patients who receive dental implant placement surgery in esthetic zone. From this pool, patients are reached by phone to be interviewed through a questionnaire for patient centered outcomes. Patients who accept the invitation receive a comprehensive clinical examination of their implant supported restorations to determine clinical outcomes. Visual Analog Scale (VAS), Pink Esthetic Score (PES), White Esthetic Score (WES) are used in addition to routine clinical parameters to evaluate peri-implant tissues.

Results: 42 subjects completed tele-questionnaire. A subgroup (18 patients; mean 64±11 years; 12 female; 33 implants; time in function 3±2 years) received clinical evaluation. Treatment time between implant placement to restoration delivery was 12±5 months (5-22) with sites receiving pre-implant bone grafting. Tele-questionnaire revealed mostly positive patient centered outcomes with a median VAS of 10 (ranging 2 to 10). Some changes in peri-implant tissue contours and increasing gingival recession on adjacent teeth were reported by 2 (5%) and 4 (10%) patients, respectively. Interproximal spacing was noticed by 4 (10%) patients. 7 (17%) patients reported unsatisfaction with the contours of their gingiva around the implant. Clinical diagnosis was 22 (67%), 10 (30%) and 1(3%) peri-implant health, mucositis and peri-implantitis sites, respectively. Median PES and WES were 9 (4-12) and WES 8 (5-10), respectively.

Conclusion: Preliminary data analysis reveals that PES and WES agree with VAS. Changes in gingival tissue contours together with space forming and/or food impaction are the main factors affecting patient centered outcomes. Recruitment and data collection are ongoing.
Environmental Impact of Saliva on Oral Streptococci Growth and Biofilm Formation

Allen Choi, Kevin Dong, Jordan Batagower and Justin R. Kaspar

**Objectives:** Oral streptococci are associated with oral disease and are integral to the formation of supragingival biofilm communities. Commonly, lab-based growth media (tryptone yeast extract with glucose; TYG) is used for oral bacterial research. However, less is known about saliva-supplemented media's effects on oral streptococci. In this study, the growth and biofilms of health- and disease-related oral streptococci species were compared in lab-based media and media with human saliva to assess saliva's effects on bacterial growth and biofilm development.

**Methods:** The growth of oral streptococci species were measured using a Bioscreen C system in TYG and compared to water-diluted and saliva-supplemented TYG media. Additional growth curves were used to compare TYG media supplemented with various saliva concentrations. Biofilm formation and cell biomass were analyzed in lab-based TYG and saliva-supplemented TYG using fluorescent microscopy.

**Results:** We determined that three of eight oral streptococci species displayed a faster doubling time in saliva-supplemented TYG, with all but one species showing faster growth in saliva than diluted TYG media. The analysis on different saliva concentrations in media yielded evidence that saliva in some capacity other than 100% correlated with an increase in growth except in *Streptococcus sanguinis*. Biofilm formation by oral streptococci was, however, limited in the presence in saliva with a concurrent decrease in biomass.

**Conclusions:** This study correlates the presence of saliva in growth media with an increase in oral streptococci growth and decrease in biofilm development. The data underlines a need to further investigate saliva's effects on oral streptococci and saliva's importance in future investigations. This project's next steps will relate to examining the components of saliva and their individual effects on oral streptococci growth and biofilm development. By studying the impacts of growth media on microbes and intermicrobial communities, we can gain more knowledge on the oral environment and improve on emulating it in *in vitro* conditions.
Sclerostin Ablation Improves Alveolar Bone Healing Following Tooth Extraction

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Objectives: Bone modeling, remodeling, and healing are regulated by the actions of osteoblasts and osteoclasts, both responding to signals from osteocytes. Sclerostin (Gene: Sost; protein: SOST) is expressed by osteocytes and regulates bone formation by inhibiting osteoblast Wnt signaling. Deletion of SOST in mice (Sost-/-) results in increased bone mass. We aimed to compare effects of Sost ablation on alveolar bone vs. long bone formation and evaluate if its absence improved alveolar bone healing. We hypothesized absence of SOST would improve alveolar bone healing.

Methods: Femurs and mandibles from Sost-/- and wild-type (WT) mice were harvested at 6-weeks-old and analyzed by micro-computed tomography (micro-CT). Maxillary first molars were bilaterally extracted from 6-week-old Sost-/- and WT mice. Tissues were collected at 0 and 21 days-post-extraction (dpe) with n=3-4/group/time point. Healing was analyzed by micro-CT and histology (H&E, Masson’s trichrome, and tartrate-resistant acid phosphatase).

Results: Effects of Sost ablation were compared in femurs vs. alveolar bone. Femurs showed increased cortical bone volume fraction and cortical thickness (25-30% each; P<0.05) in Sost-/- vs. WT mice. While total alveolar bone volume was not significantly altered, alveolar bone proper (most closely related to tooth attachment) and mandibular bone volumes were increased (50% and 30%, respectively; P<0.05) in Sost-/- vs. WT mice. We tested the effect of Sost ablation on alveolar bone healing after molar extraction. At 21 dpe, micro-CT revealed increased bone volume fraction (20%; p<0.05) in Sost-/- vs. WT mice. Compared to WT, Sost-/- mice showed more mature, compact bone, less marrow space, and enhanced collagen organization. Osteoclast numbers were not different between genotypes.

Conclusions: Ablation of SOST promoted increased femur cortical bone, alveolar bone proper, and mandibular bone volumes. Absence of SOST contributed to more rapid, improved socket healing following tooth extraction, suggesting a potential therapeutic strategy targeting SOST to promote alveolar bone maintenance or periodontal regeneration.
Machine Learning with Cross-Institutional Prediction for Orthodontic Tooth Extraction

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Abstract: The objective of this study was (1) to determine the performance of machine learning in prediction of extraction vs non-extraction for orthodontic patients from two university data sets and (2) to identify and compare rankings of most important features.

Methods: Subjects consisted of 297 patients in the Graduate Clinic of Orthodontics at OSU for orthodontic treatment during a consecutive enrollment from 2017-2020. Input features (9 clinical, 11 cephalometric) were identified based on previous studies. Random forest (RF) model was trained using these feature sets on the sample population. The performance of each model was evaluated using measures including sensitivity, specificity, balanced accuracy, accuracy, positive predictive value (PPV) and negative predictive value (NPV). RF models trained by OSU data (OSU model) and previously obtained UNC data (UNC model) were used to predict on both OSU data and UNC data. Feature rank was calculated by RF to determine importance of each feature in extraction vs non-extraction decision.

Results: The sensitivity and specificity were 0.47 and 0.97, respectively, for OSU model and 0.32 and 0.94, respectively, for UNC model. Cross-prediction of data shows decrease in sensitivity for OSU model predicted on UNC, but similar sensitivity for UNC model predicted on OSU. Maxillary and mandibular crowding were the two most important features in both institutions.

Conclusion: The extraction vs non-extraction decision is a difficult challenge orthodontists face in clinical practice. AI expert system could provide new insight in addressing this long-standing debate. This study advanced the research in the field by applying it to multiple data centers in the US. Different philosophies/beliefs may exist in different institutions, but major factors are the same. Therefore, it is promising to use AI to predict results from different centers.
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**Accuracy of Miniscrew Placement in Maxilla Using Virtual Reality Approach**

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**Introduction:** One of the most substantial disadvantages of TADs is the high failure rate when compared to dental implants. The major reason for failure of miniscrews is root proximity and root contact. Currently, the use of panoramic radiographs and cone beam CT (CBCT) images are the primary methods for planning the site of TAD placement. Recently, the use of virtual reality (VR) software has become more popular in medicine and dentistry for the planning of surgical procedures. We believe these 3-dimensional VR models may be useful in planning for TAD placement.

**Methods:** VR models were rendered from CBCT images of cadaver canine maxillae using Immersive View software. One group of orthodontic residents/dental students treatment planned for TAD placement using the Immersive View VR program while another used CBCT images. After treatment planning, both groups placed TADs in the cadaver dog maxilla. The miniscrew to root proximity were measured and compared using CBCT between two groups.

**Results:** Average mesial root-miniscrew distance was 2.73 mm (SD of 1.10) for the VR group and 2.78 mm (SD of 1.94) for the CBCT group. Average distal root-miniscrew distance was 3.07 mm (SD of 0.83) for the VR group and 3.10 mm (SD of 1.13) for the CBCT group. On average, there is no statistically significant difference of the interradicular distances for the VR and CBCT groups (p-value=0.898). On average, there was no statistically significant difference was observed between the VR and CBCT groups for the mesial root-miniscrew distances (p-value=0.7975) or the distal root-miniscrew distances (p-value=1).

**Conclusions:** The hypothesis that there was a significant difference in the accuracy of miniscrew placement between those using CBCT and VR before placement was rejected. However, VR training may have the benefit of providing visual and tactile feedback to inexperienced users learning to place miniscrews.
Osteopontin Ablation Partially Rescues Cellular Cementum Hypomineralization in Osteomalacic Mice

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Abstract: X-linked hypophosphatemia (XLH) is an inherited disorder characterized by hypophosphatemia, renal phosphate wasting, rickets and osteomalacia caused by loss-of-function mutations in the PHEX gene. Hypophosphatemia in XLH is associated with mineralization defects in dental tissues including interglobular dentin dysplasia, wide pulp chamber, and hypomineralized cementum, leading to spontaneous dental abscesses, periodontal diseases, and tooth loss. The pathophysiology of these defects remains poorly understood, preventing effective treatments. PHEX is expressed in cementocytes, whereas its deletion leads to accumulation of osteopontin (OPN) in cellular cementum, suggesting OPN may contribute to XLH-associated dental symptoms. We hypothesize that OPN ablation in the Hyp mouse model could rescue cellular cementum defects. We compared 50-day-old Hyp;Spp1/- double-knockout (dKO) mice with WT, Spp1/-, and Hyp mice on a normal and high phosphate (HP) diet, focusing analysis on first mandibular molars (M1). On normal diet, histology of M1 showed no difference in cellular cementum area among experiment groups; yet, Hyp mice exhibited higher cementoid/cementum ratio, indicating cellular cementum hypomineralization. Interestingly, dKO molars showed significantly reduced cementoid/cementum ratio (48%) compared to Hyp mice, suggesting improved mineralization. However, OPN ablation in Hyp mice did not completely rescue XLH-associated periodontium/cellular cementum defects on normal diet. Compared with normal diet, mice on HP diet exhibited overall improvement in dental mineralization. MicroCT of M1 showed no difference in cellular cementum volume among genotypes. While significantly improved by HP, cellular cementum in dKO and Hyp mice showed 4% remaining density defects. For cellular mechanisms, histomorphometry of silver-stained cementocytes on both diets failed to show differences in cementocyte density, lacunar area, height and width, due to inherent variations of cementocyte size. We conclude that OPN ablation partially rescued XLH-associated cellular cementum hypomineralization, yet OPN is not the major contributor to XLH pathophysiology and alternative mechanisms/approaches should be considered.
Decrease of Cervical Vertebral Bone Density in Women with Aging

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Objectives: Postmenopausal osteoporosis is a systemic bone disease in women with aging. If left untreated, patients with this disease often experience pathologic fractures among other morbidities. Diagnosing osteoporosis has traditionally been conducted by assessing bone mineral density (BMD) of thoracic or lumbar spine using 2-dimensional imaging of dual energy X-ray absorptiometry (DXA). Otherwise, the objective of this study is to examine whether dental 3-dimensional cone beam computed tomography (CBCT) can assess BMD changes of the cervical vertebrae as an alternative diagnostic tool.

Methods: Following IRB approval, 186 CBCT images were retrospectively obtained from 64 male and 122 female patients (20 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) was digitally isolated by removing posterior and lateral processes. A gray value, which is proportional to BMD, was assessed. Mean gray values of C2 were computed and tested using one way analysis of variance with Tukey post hoc test 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 the female 40-and 50-age groups (p<0.001) while it was not significantly different between all other age groups in male and female groups (p>0.22). The mean gray values of the female group significantly decreased with age (p<0.001) but those of the male group did not (p=0.077).

Conclusion: Menopause of the female 60-age group likely results in significantly lower BMD values compared to the young female group. These findings suggest that the CBCT based C2 BMD could be a potential diagnostic tool for osteoporosis with aging.
Inhibitory Neuron Subpopulations in the Gustatory NST

Emma Gutarts, Charlotte Klimovich, Keerat Sandhu, and Susan Travers

Objective: The rostral, gustatory nucleus of the solitary tract (rNST) contains many inhibitory, GABAergic neurons. In other central structures, inhibitory neurons are varied with phenotypically-defined populations having different functions. The objective of the present study was to begin to determine the degree to which there are different inhibitory neuron subpopulations in the rNST.

Methods: The present study used paraformaldehyde-fixed, cryostat-sectioned (15μm) sections from C57BL/6 mouse brains (N=5F,5M) triple-labeled using in situ hybridization for different combinations of markers for GABA (inhibitory) and glutamate (excitatory) neurons and for neuropeptides. GABA markers included a synthetic enzyme for GABA, glutamate decarboxylase 65 (GAD65), and the vesicular transporters (VGAT); the excitatory marker was the vesicular glutamate transporter, VGLUT2. The neuropeptide probes were for somatostatin (SST) and preproenkephalin (PENK), a precursor for enkephalins.

Results: There was almost complete overlap between expressions of VGAT and GAD65. As we observed previously using co-staining with VGAT (Kalyanasundar et al, 2022), less than one-third of rNST inhibitory neurons stained for SST. However, many additional inhibitory neurons co-expressed PENK. Interestingly, although a majority of both SST (~60%) and PENK (~70%) neurons were inhibitory, substantial subsets of both these peptidergic populations expressed VGLUT2, suggesting that these peptides are also in excitatory neurons.

Conclusion: Because VGAT is always present in GABA neurons, this suggests that, unlike in other neural regions such as the olfactory bulb, GAD65 does not define a limited subset of inhibitory neurons. However, in contrast, the peptide staining suggested that there are distinct subsets of inhibitory cells since many GABA neurons expressed just one or neither peptide, rather than both. Future studies could explore the behavioral effect of manipulating these different inhibitory cells. Moreover, it would be interesting to determine the significance of the apparent co-expression of excitatory (glutamate) and inhibitory (PENK) transmitters.
Determining Potential Correlation between Streptococcus mutans Microcolony Size and Acidity

Delaney Hancock, Madisen Bangs and Justin R. Kaspar

Objectives: *Streptococcus mutans* (SMU) utilizes dietary carbohydrates to produce organic acids which demineralizes the tooth enamel. Our lab has an isolate bank of SMU strains from around the world that display phenotypic diversity. One aspect we aimed to explain was variance in microcolony size observed between strains during biofilm formation. The purpose of this study was to determine if differences in microcolony size was a main factor for differences in acid accumulation produced between strains.

Methods: Biofilms of ten different SMU isolates were grown in TYG for 24 hours. At the 24 hour mark, a fluorescent dye, pHrodo, was used to stain the biofilms prior to imaging. A Biotek LionHeartFX automated microscope was utilized to image and measure both the fluorescent intensity of the pHrodo dye, as well as the volume of each microcolony within the biofilm between strains.

Results: Utilizing the microscopy images and measurements, we found no correlation between the acidity of the microcolony based on its fluorescent intensity and the microcolony volume. However, we did find one outlier, SMU107, which showed significant acidity and volume compared to the other isolates.

Conclusion: Data obtained from this study did not yield any significant evidence that SMU strains that produce larger microcolonies are also more acidic. However, SMU107’s contrasting differences from the other isolates warrants further investigation. By studying the differing behavior of SMU strains, we main gain further insight into the virulence potential of the species and how phenotypic diversity impacts disease outcomes and therapeutic interventions.
Effects of Estrogen Deficiency on Jawbone of Aged Rat

A. Hassouna, J. Liu, M. Kim, D. Kim

Objectives: Postmenopausal osteoporosis is developed due to estrogen deficiency, which decreases bone mineral density (BMD) of orthopedic bone. It is unclear whether this alters BMD of jawbone as well. The objective of this study was to investigate whether estrogen deficiency has an effect on tissue mineral density (TMD) distribution in the mandible using an ovariectomized (OVX) rat model.

Methods: Following approval of IACUC protocol, 15 female Sprague Dawley rats including 10 sham (3 months following sham operation at 6 months old) and 5 aged OVX (2 months following OVX surgery at 12 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 (Low) and high (High) TMD for each mandible. One way analysis of variance was performed to compare the TMD parameter values between sham and aged OVX groups and Pearson's correlation was also tested. A significant level was set at p<0.05.

Results: Most of the TMD parameters were not significantly different between the young sham group and aged OVX group (p>0.08) while the High values of WB and AB were significantly higher for the aged OVX group that the young sham group (p<0.02).

Conclusions: The current findings indicate that the TMD distribution of jawbone is not altered by aging and estrogen deficiency. The increase in the High, which results from removal of pre-existing bone tissues, is likely compromised by active bone formation and mineralization to maintain other TMD parameters.
Comparison of machine learning methods in dental crowding predictions

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

Objective: This study compares the accuracy of a self-supervised machine learning (ML) method with previous methods in analyzing maxillary dental crowding using clinical intraoral photos.

Background: Convolutional neural networks (CNN), a type of ML algorithm, have been used in medical image analysis. These algorithms have also been used in orthodontics for clinical measurements, treatment planning, and diagnosis. Previous research has shown CNN to be a promising alternative to manual measurements of dental images, with some limitations preventing its application. Transfer learning, initializing the CNN's parameters from other trained models, has been widely used in CNN. Thus, this study compares the accuracy of CNNs using a self-supervised pretrained method to a public natural-image-domain database pretrained method (ImageNet-pretrained) to predict maxillary crowding from intra-oral images.

Methods: Maxillary crowding was measured by orthodontic residents on 832 patients and intraoral photos were taken. Using residual neural network (Resnet) as the backbone, SimCLR, a self-supervised learning method, was pre-trained on maxillary images to develop its base parameters, as were an ImageNet-pretrained method and a non-pre-trained method. Each method was trained and validated on 665 maxillary images. The well-trained models predicted the remaining 167 crowding values. These predictions were compared to the clinical crowding measurements crowding. Each model's error was compared to identify the greatest accuracy.

Results: The traditionally pretrained ImageNet method resulted in a Resnet model with the lowest error, while the SimCLR pretrained model returned the second lowest error. The model with no pretrained returned the greatest error.

Conclusions: The model presented in previous research, ImageNet pretrained Resnet, remained the best of the currently tested methods for predicting maxillary crowding values from photos. However, the self-supervised pretrained model showed a similar accuracy using far less number of images in the pretrained phase, indicating its potential to reduce the required number of samples in ML.
The Effects of Osteopontin on Dentin Regeneration Following Tooth Injury

Morgan Horvath, Irene Kim, Lauren Wilks, and Sarah B. Peters

Objective: Osteopontin (OPN), or secreted phosphoprotein (Spp1), is a major extracellular matrix protein expressed by odontoclasts and odontoblasts and has been shown to be crucial for bone and dentin mineralization. Following injury or infection, odontoblasts secrete tertiary dentin to protect the soft, pulpal tissue and strengthen the mineralization and integrity of damaged areas. We hypothesize that OPN−/− mice will demonstrate reduced dentin regeneration after injury compared to WT mice.

Methods: To test our hypothesis, we used a #1/16 bur to injury the dentin of first mandibular molars on the mesial side in WT and OPN+/− mice and allowed 21 days of recovery. Pulp tissue was not exposed at any time. We performed microcomputed tomography (micro-CT) on the injured molars at 6 µm resolution and histopathology with hematoxylin and eosin (H&E) staining. Uninjured control mice were included for both genotypes.

Results: Preliminary evidence showed dentin regeneration in H&E images of WT molars 21 days after injury and none in the OPN+/− molars. Micro-CT imaging was unable to detect tertiary dentin in both WT and OPN+/− molars.

Conclusions: Our histopathology supports the hypothesis that OPN−/− mice demonstrate reduced in dentin regeneration after injury in comparison to WT mice. This leads us to believe that OPN is necessary for dentin regeneration. Future directions include micro-CT imaging at higher resolution and immunofluorescence of mineralization markers with fluorescent microscopy. Longer recovery times will also be included to investigate more thorough tissue repair. These experiments will help determine whether dental therapeutics with OPN supplements could improve restorative dental outcomes.
Alveolar Bone Mineral Density Decreases in Aging Women

O. Jackson, V. Sabato, F. Chaudhry, K. Doan, D. Kim

Objectives: Women have a higher risk of periodontal disease and tooth loss with aging than men. It was also found that women with osteoporosis and osteopenia may exhibit greater loss of clinical attachment, leading to periodontal disease and tooth loss. While postmenopausal osteoporosis and osteopenia show a higher risk of decreased bone mineral density (BMD) and bone loss in orthopedic bones of women, their effects on jawbones have not been extensively evaluated. The objective of this study is to examine whether BMD of the jaw changes in men and women with aging.

Methods: Following IRB approval, cone beam computed tomography (CBCT) images were retrospectively obtained from 103 patients. Forty-eight males (15 to 82 years) and 55 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). 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 with post-hoc Tukey test and Pearson’s correlations were examined for aging effects on BMD.

Results: The 40-age female group had significantly higher AB BMD value than the 50- and 60-age female group (p<0.05) but the AB and CB BMD values were not significantly different between all other groups (p>0.1). The AB BMD significantly decreased with age in combined male and female groups (p<0.01) but the CB BMD did not decrease with age (p>0.5).

Conclusions: The current results show that BMD of AB is decreasing in aging women. As AB directly surrounds the teeth, this finding suggests the increased risk of periodontal disease and tooth loss in aging women.
Bone Mineral Density of Temporomandibular Condyle in Dental Implant Patients

Nathan Kim, Ian Segall, Zachary Skabelund, Sonya Kalim, Lisa Knobloch, Hany Emam, Do-Gyoon Kim

**Introduction:** Temporomandibular joint osteoarthritis (TMJOA) is an important subtype of TMJ disorders (TMD) which can cause pain and dysfunction of the joint. While TMJOA is diagnosed in all populations and age groups, the exact etiology for this multifactorial joint disease has not been fully elucidated. The only consensus is that TMD is more prevalent in women than men. The objective of this study is to examine whether replacement of teeth with dental implants has an effect on the risk of TMJOA.

**Methods:** Following IRB approval, cone beam computed tomography (CBCT) images were obtained retrospectively from 301 patients. A total of 186 patients with an implant-supported occlusion (90 patients including 46 Men, 63.26±8.74 years old and 44 Women, 62.00±7.60 years old) and without (96 patients including 52 Men, 64.8±9.56 years old and 44 Women, 65.3±8.31 years old) was identified. TMJ condyles were digitally isolated from the images. A histogram of gray level that is proportional to bone mineral density (BMD), was obtained from each image. Analysis of variation (ANOVA) with post-hoc Tukey test was conducted to compare the BMD of TMJ condyle between men and women with and without implants.

**Results:** Women had significantly higher BMD values than men without an implant (p<0.001) but it was not significant between those with an implant (p=0.24). Men with an implant had significantly higher BMD values than those without an implant (p=0.026) but it was not significant between women with and without an implant (p=0.9).

**Conclusion:** These findings indicate that dental implantation likely alters BMD of TMJ condyles. Future studies including more CBCT images are currently being performed.
Aging Effects on Mature Rat Mandible

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Objectives: The most widely used pre-clinical model of post-menopause is an ovariectomized (OVX) female rat model. Six month-old rats are preferred for the OVX model because bones of a normal rat rapidly grow to be skeletally mature by 6 months of age but aging effects begin at 12 months. A lack of knowledge exists for the aging effects on jaw bone of the rat. Thus, the objective of this study was to examine whether mineralization and morphology of mature jaw bone changes with normal aging.

Methods: Following IACUC approval, 36 Sprague–Dawley female rats (7 for 9 months, 6 for 9.5 months, 7 for 10.5 months, 9 for 12 months, and 7 for 15.5 months) were obtained. The mandible was dissected and scanned using micro-computed tomography at 27-micron voxel size. A heuristic algorithm was used to segment mineralized tissue voxels from other voxels. Cortical bone (CB) and a trabecular bone (TB) were digitally separated by the process of compartmentation. Alveolar bone (AB) was segmented within 200 micron from the teeth surface. The CT attenuation value of each bone voxel, which is proportional to bone tissue mineral density (TMD), was calibrated with known density phantoms. A histogram of TMD was obtained to compute mean, standard deviation (SD), lower and upper 5th percentile (Low5 and High5) values. TB morphological parameters were also computed. Correlations of age with the TMD and morphological parameters were tested.

Results: Age had a weak but significant positive correlation with TB TMD Low5 (p=0.03) but a negative correlation with TB High5 (p=0.02) resulting in more homogeneous TMD distribution with lower SD (p<0.001). All other correlations with age were not significant (p>0.1).

Conclusion: The current findings indicate that the bone mineral and morphology characteristics of jaw bone in mature rats are not sensitive to aging.
Environmental Impact of Treating Pediatric Dental Caries

Lee J., Hammersmith K., Townsend T., Meyer B.

**Purpose:** The United States healthcare sector is a significant contributor to national greenhouse gas emissions which adversely affects environmental, overall, and oral health. The healthcare system has the obligation and the opportunity to evaluate resource use, reduce waste, and decrease environmental burden, through actions such as lessening the incidence of preventable diseases, e.g. dental caries. Specifically in dentistry, the limited environmental research available demonstrates that patient travel emissions, nitrous oxide use, and product procurement are among the highest contributors to the industry’s environmental burden. The aim of this study was to estimate the care-associated carbon footprint for a treatment visit for a child with early childhood dental caries.

**Methods:** Data was collected on children 6 years and younger seen in the hospital dental clinic. Preventative visits (exam, prophy) were not included. Visit types were categorized by procedures and integrated with single-use plastic (SUP) material data collected from the procedure set-up per hospital guidelines. Visit data for treating dental caries, including nitrous oxide use, distance traveled by family, visit type and thus single-use materials, were collected. Travel and nitrous data were converted into carbon equivalents and assessed.

**Results:** Data from 559 patients with dental caries was included. The patients, on average, traveled 50.8 miles round-trip for a visit, contributing 20.45 kgCO2e. On average, 61.12 SUP were utilized per visit. Forty-six percent of treatment visits utilized nitrous oxide for a mean duration of 20 minutes contributing 26.8 kgCO2e.

**Conclusions:** Patients with dental caries, on average, contribute 47.25 kgCO2e and 61.12 SUPs per dental treatment visit.
Predicting Invisalign Treatment Time Using Machine Learning

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Objectives: The objective of this study is to validate the ability of a machine learning algorithm to predict aligner treatment time.

Methods: A total of 69 Invisalign cases treated at The Ohio State University graduate orthodontic clinic were selected. The initial and final digital scan were superimposed, and an in-house imaging AI program was used to segment teeth and construct a 3D coordinate system for each tooth. The 3D coordinate system presents the 6 types of tooth movement or degrees of freedom (DOF) per tooth for a total of 12 maxillary teeth. A total of 14 independent variables (simplified DOF per patient (6 variables), maxillary only PAR score (6 variables), gender and age were introduced into the random forest algorithm for a binary classification into two categories: short treatment (≤12 months) or long treatment (>12 months).

Results: Predicting the exact treatment time using regression analysis yields unsatisfied results ($R^2 = -0.48$); however, Random Forest classifier was able to successfully estimate treatment time by distinguishing between two categories: short treatment (<12 months) and long treatment (>12 months) with a 67% accuracy. The performance of our classification model had 0.81 sensitivity (long) and 0.44 specificity (short). In addition, results suggest that patients age, extrusion/intrusion, mesial/distal and tipping tooth movements might play an important role in treatment time prediction.

Conclusions: Predicting the exact treatment time in months using random forest regressor is very challenging, however, Random Forest can classify between short treatment and long treatment with a 67% accuracy. Results suggest that there is promise in using machine learning for treatment time predictions.
3D Reconstruction from 2D-Panoramic X-ray to Assess Maxillary Impacted Canines

Sumeet Minhas, Tai-Hsien Wu, Do-Gyoon Kim, Ching Chang Ko

Abstract: The objective of this study was to verify if 3D reconstruction is feasible to assess maxillary impacted canines from a 2D panoramic X-ray.

Introduction: Maxillary canines are the second most common teeth after third molars to be impacted in the dental arch. To determine the position and spatial context of the impacted canine, radiographic assessments are used with either 2-dimensional radiographs (panoramic X-ray and lateral cephalometric) or 3-dimensional imaging technology (cone beam computed tomography [CBCT]). Leveraging the deep-learning reconstruction technique, we hypothesize that we will be able to reconstruct the 3D dental structure and tooth position from the 2D panoramic X-ray.

Methods: Pre-treatment CBCT data for 74 patients with impacted canines were collected. A dataset containing paired 2D panoramic X-rays and pseudo 3D images were converted from all 74 subjects. These data were used to train a deep-learning reconstruction algorithm. The location (buccal, palatal, middle) of the maxillary impacted canine was determined from the output of the algorithm. The reconstruction was evaluated using structure similarity index measure (SSIM) as a metric to indicate the reconstruction quality. Additionally, the accuracy of the impacted canine location was calculated.

Results: Preliminary results show a 44% accuracy of the reconstruction algorithm in predicting the position of the impacted canine. The mean SSIM for the current output is 0.75 with a range from 0.7-0.8.

Conclusion: Although our SSIM values were equivalent to the values reconstructed by other deep-learning algorithms in the dental field, we found that our 3D reconstruction had less than 50% accuracy in determination of the impacted canine location. Further clinical verification is needed to validate the robustness in dental reconstruction applications.
Alpl Ablation in Dental Epithelium Disrupts Ameloblasts and Enamel Mineralization

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Background: Tissue-nonspecific alkaline phosphatase (Gene: ALPL; protein: TNAP) is a mineralization-associated enzyme that hydrolyzes mineralization inhibitor pyrophosphate. Alpl/TNAP is highly expressed in maturing ameloblasts and the underlying stratum intermedium layer of enamel epithelium. Loss-of-function mutations in ALPL cause wide-ranging tooth abnormalities including hypoplastic and/or hypomineralized enamel in the inherited disease, hypophosphatasia (HPP). HPP-associated enamel defects are phenocopied in Alpl-deficient mice; however, the short lifespan and severe systemic effects have hampered efforts to understand the direct role of Alpl/TNAP in enamel formation.

Methods: Alpl was conditionally ablated in enamel epithelium by crossing Krt14-Cre with Alplfl/fl mice to develop Krt-14-Cre;Alplfl/fl conditional knockout (cKO) mice. We analyzed cKO and control (CTR; Alplfl/fl) tissues at 14 and 60 days postnatal by serum biochemistry, micro-computed tomography (micro-CT), histology, in situ hybridization (ISH) and immunohistochemistry (n= ≥ 6/group).

Results: Compared to CTR, cKO mice showed no differences in body weight, serum alkaline phosphatase, or cranial and appendicular bones parameters, demonstrating no systemic effects. Compared to CTR, cKO teeth displayed white lesions on incisors, worn incisal edges, and flattened molar cusps, more evident in cKO males than females. Micro-CT indicated no significant differences in enamel, dentin, or bone volumes in cKO vs. CTR. Compared to CTR, cKO incisors showed reduced enamel density, affecting males (18%) more than females (1.4%), with no differences in thickness of unerupted enamel. Histology of incisors revealed impaired amelogenesis in pre-maturation ameloblasts due to ameloblast/enamel detachment near the enamel-dentin junction, aberrant proliferation, disorganized multicellular layers, and deposition of an amorphous substance. ISH showed comparable amelogenin expression in cKO vs. CTR incisors; however, immunohistochemistry showed aberrant amelogenin staining in cKO ameloblasts.

Conclusion: Conditional Alpl ablation in enamel epithelium reveals a role in maintaining ameloblast phenotype and enamel mineralization, providing a model to study underlying mechanisms of HPP-associated enamel phenotypes and to improve our understanding of amelogenesis.
Analysis of the dentin matrixsome composition of female rats over time

Mona Omar, Michelle Blyumin, Sarah B. Peters

Objective: In response to injury/infection, teeth undergo a strong repair process, including neurite outgrowth. This is believed to be in response to the release of neurotrophins released from the dentin during the process exhibiting outgrowth. This project aims to determine whether the age-related changes in dentin include changes in growth factor contents that would affect the regenerative capacity of molars.

Methods: Molars were removed from maxillae and mandibles from 4 young (3 months old) and 4 old (24 months) female rats. The crown and roots were separated, and pulpal tissue was removed. The mineralized portions were then cryomilled, dentin matrix proteins were extracted, and proteomic analysis was performed at the OSU Proteomics Shared Resource.

Results: Both old and young female molars had more proteins identified in the roots than the crowns. We divided the proteins into six groups: collagens, extracellular matrix (ECM)glycoproteins, proteoglycans, ECM-affiliated proteins, and ECM regulators. We discovered 11 upregulated proteins in young root females and 82 upregulated proteins in old root females. The old roots female contained 48 upregulated BPIDs compared to the young roots.

Conclusion: Our findings show that the crowns and roots of female rat molars undergo age-related changes, and they provide a solid basis for developing precision-based regenerative treatments for vital pulp treatments.
Background and Objectives: Periodontal disease progression rate is determined based on the amount of bone loss in relation to patient’s chronological age. However, limited information is available on the prevalence of bone loss in adolescence and early adulthood. This retrospective study aims to determine the prevalence of bone loss in patients from the age of 15 to 65 yrs (10 different age brackets in five-year increments from Group 1 [G1] to Group 10 [G10]).

Material and Methods: Patients dental radiographs documented at their initial visit to The Ohio State University College of Dentistry through access to AxiUm e-chart system are used to determine bone levels (IRB protocol #2022H0050). A training exercise is conducted among four clinicians for radiographic evaluation prior to data collection. Presence of bone loss is defined as ≥2.5mm vertical space from CEJ to alveolar crest between two natural contiguous maxillary and/or mandibular posterior teeth by examining bitewing radiographs. If present, the amount of bone loss is recorded. Demographic information including patient’s age and gender, and clinical dental/periodontal examination at the time of initial visit are also reviewed. Data collection is ongoing.

Results: 900 charts were screened and 436 charts with acceptable radiographic documentation were included into this preliminary data analysis. Sample numbers for each age bracket were as follows: G1: 22 (15-19 yrs), G2: 65 (20-24 yrs), G3: 64 (25-29 yrs), G4: 52 (30-34 yrs), G5: 39 (35-39 yrs), G6: 40 (40-44 yrs), G7: 34 (45-49 yrs), G8: 42 (50-54 yrs), G9: 38 (55-59 yrs), G10: 49 (60-65 yrs). 32% of the study population presented radiographic bone loss (ranging from 3 to 7 mm). The percentage of patients with bone loss was 0, 5, 6, 21, 36, 38, 56, 43, 79 and 53% for G1 through G10, respectively. The number of missing teeth increased with increasing age. Recruitment and data analysis are ongoing for this multicenter study.

Conclusion: The results of this study may be significant in providing information related to onset of periodontal bone loss at posterior sextants. Specific risk assessment tools used in dental training settings can be accordingly improved based on this data.
Predictors of Burnout or Intention to Leave the Dental Hygiene Profession

Johanna Owen, Rachel Kearney

Introduction: Dental hygiene is a demanding profession requiring physical and mental work in a clinical setting. Because of the demands of the professional role, dental hygienists are susceptible to burnout.

Purpose: The purpose of this study is to determine the predictors of burnout in dental hygienists and their intention to leave the profession.

Methods: This is a quantitative cross-sectional research study. The study instrument consisted of The Maslach Burnout Inventory-Health Services Survey distributed to dental hygienists via social media platforms.

Results: The responses of 131 dental hygienists were used. Mean scores for emotional exhaustion showed there was a significant relationship between intention on leaving the dental hygiene profession in the next 5 years and emotional exhaustion (p=0.0001). There was a significant difference in emotional exhaustion between those who intend to leave the profession and those who do not (p < 0.0001) and between subjects that maybe want to leave the profession (p = 0.006).

Conclusion: Dental hygienists who responded to this survey are experiencing moderate burnout in the categories of emotional exhaustion and depersonalization. Personal accomplishment showed lower levels of burnout. The main predictors of burnout are linked to if the dental hygienist is intending to leave in the next 5 years, hours worked, and patients that are seen per day.
Accuracy of Implant Placement with 3D-Printed and Conventional Surgical Guides

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Objective: Conventional thermoplastic surgical guides exhibit higher flexure, provides limited details on supporting tissue topography and are less accurate in guiding the implant to its ideal position – limitations that digital 3D-technology (3DG) may be able to overcome. The aim of this clinical study is to evaluate the accuracy of dental implant placement utilizing CAD/CAM technology compared to a conventional thermoplastic surgical guide.

Material and Methods: Patients that are planned for single-unit implant placement therapy with intact adjacent teeth are recruited. Inclusion criteria: systemically and periodontally healthy/stable. Exclusion criteria: medically compromising conditions/medications that are contraindicated for implant surgery, completely edentulous or do not have enough existing teeth for a tooth-supported surgical guide, no indication for tomography (CBCT) documentation. The ideal implant position is determined by fabricating a virtual implant plan through software, digital impressions and CBCT. Implant stability is measured at the time of implant placement and 4-6 months post-operatively with Resonance Frequency Analysis (RFA). Deviation in planned implant location is determined by superimposing post-surgical digital implant impression and initial virtually placed implant fixture by software.

Results: 22 patients (mean age 58 yrs [20-75 yrs]; 8 male; 15 implants) were recruited (11 CTG and 10 3DG) with single case excluded due violation of post-operative study protocol. Seven sites were previously bone grafted (5 in CTG and 3 in 3DG). Mean initial ISQ values were 63±7 and 68±9 for CTG and 3DG, respectively. Changes in implant stability following 4 months healing period was negligible. Preliminary data reveals minimal deviations from virtually planned implant placement for both groups. Recruitment/data collection is ongoing.

Conclusion: There is no significant difference in dental implant placement accuracy with the use of a CAD/CAM 3D-printed surgical guide compared to a conventional thermoplastic surgical guide when distal and mesial tooth supports are present. (Supported by The Divisions of Periodontics and Prosthodontics at The Ohio State University, College of Dentistry)
Subgingival and salivary microbiota in placenta drive pregnancy complications

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Objectives: Infection-driven inflammation is a leading cause of pre-term birth, however, local versus hematogenous infection is poorly studied. Therefore, this investigation aimed to interrogate the subgingival and salivary microbiomes as potential sources of placental microbiota.

Methods: Saliva, plaque, serum, and placenta were collected from 130 women who delivered either full-term (FT), pre-term (PT) or pre-term complicated by pre-eclampsia (PTPE). Whole-genome shotgun sequencing, reverse transcriptase real-time PCR, and source tracking were employed to (i) investigate discriminants of pregnancy outcomes, (ii) measure placental immuno-inflammatory response, (iii) explore hematogenous spread as a factor in pregnancy complications, and (iv) interrogate subgingival and salivary microbiomes as sources of placental microbiota.

Results: The placental microbiome cannot be attributed to environmental contamination, sequencing, or delivery mode (p=0.973, PERMANOVA). Placental microbiota and TLR responses are discriminants of pregnancy complications, outweighing hypertension, BMI, smoking status, and maternal age. Aggregatibacter actinomycetemcomitans and Enterococcus durans were enriched in PT while Granulicatella elegans, Tannerella forsythia, Treponema denticola, Prevotella intermedia, and Campylobacter gracilis were significantly higher in PTPE. Genes encoding quorum sensing, vancomycin resistance, cysteine, serine metabolism, iron transport were enriched in PT and PTPE. Serum was the source of placental microbiota in all 130 subjects. Subgingival plaque contributed to 6.8% of serum bacteria in FT, 12% in PT and 1.4% in PTPE groups. 15% of the serum microbiota was attributable to a non-oral source in FT and PT groups but only 3% in PTPE (p<0.05, ANOVA).

Conclusion: The oral microbiome is a significant source of placental microbiota. Translocation of oral species via serum is associated with pre-term delivery and pre-eclampsia. Both intact cells and nucleic acid are sources of microbe-associated molecular patterns (MAMPs) that upregulate placental inflammation. Our findings underscore the importance of pre-natal and natal oral health in maintaining not only maternal health, but also the health of the next generation.
Available Carbohydrates Influences Behavior of Streptococcus mutans with Commensals

Daniel Peters, Justin R. Kaspar

Objectives: *Streptococcus mutans*, associated with the development of dental caries, can efficiently utilize dietary carbohydrates and produces organic acids as a byproduct of fermentation. Previously, our group has shown that the gene expression of *S. mutans* becomes altered during growth with health-associated commensals, however the purpose of these changes and who benefits from them remain unknown. One potential hypothesis is that cross-feeding occurs between the commensals and *S. mutans*. To begin to examine this, we evaluated growth of *S. mutans* in coculture with commensal streptococci using varying carbohydrates to determine their impacts on the resulting interactions.

Methods: Monoculture growth was examined for five streptococci species using seven different carbohydrates through a Bioscreen C measuring optical density (OD) to determine sugar utilization. Coculture growth with all species was completed with UA159 pMZ (no *gfp* control) and UA159 pMZ-Pveg::*gfp* with GFP and OD recorded to determine *S. mutans* growth changes. Both experiments were done over a 24-hour period with measurements every 30 minutes.

Results: All species tested had varying growth patterns with certain carbohydrates increasing or decreasing doubling time or giving a diauxic lag phase. Others were unable to utilize specific carbohydrates. Plate reader coculture data depicted atypical *S. mutans* growth and increases in GFP values as the cocultures reached stationary phase, indicating potential cross-feeding with an unknown metabolite within these conditions.

Conclusion: The study found evidence of potential cross-feeding between health-associated commensals and *S. mutans*, supporting our hypothesis and providing context for gene expression changes during these exchanges. This investigation highlights the importance of accurately including different dietary carbohydrates to closely mimic the natural oral biome and examining coculture interactions of commensals. By examining the intermicrobial interactions with common environmental carbohydrates we could discover parameters of health and disease associated environments.
Contributions of Osteopontin to Dentoalveolar Tissues in Osteomalacic Hyp mice

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Objectives: X-linked hypophosphatemia (XLH) is an inherited disorder where gene mutations lead to loss-of-function in the enzyme PHEX. This results in elevated renal phosphate excretion and osteopontin (Gene Spp1; protein OPN) accumulation that together cause mineralization defects in bones and teeth. Genetic ablation of OPN (Spp1−/−) and increased dietary phosphate in the Hyp mutant mouse model of XLH improve mineralization defects in appendicular bone. We hypothesized that OPN ablation and high phosphate diet in Hyp mice would ameliorate XLH-associated dentoalveolar defects.

Methods: Wild-type (WT), Hyp mutant, Spp1−/−, and Hyp:Spp1−/− (double-null; dKO) mice were fed a normal or high-phosphate diet and dentoalveolar tissues were collected at 50 days postnatal (n=3/group). Tissues were analyzed by Micro-CT, histology, and immunohistochemistry for OPN, Bone Sialoprotein (BSP), and Tissue Non-Specific Alkaline Phosphatase (TNAP).

Results: Immunostaining confirmed increased OPN in dentin and alveolar bone of Hyp mutant mice, and absence of OPN in Spp1−/− and Hyp:Spp1−/− dKO mice. Micro-CT analysis showed no differences in enamel, dentin, or alveolar bone volumes or densities in dKO vs. Hyp mice. Histology confirmed similar persistent defects in dKO and Hyp mice, including wide predentin and disrupted osteocyte lacunocanalicular properties. Hyp and Hyp:Spp1 dKO mice fed a highphosphate diet showed improvement in enamel, dentin, alveolar bone, and normalized osteocyte lacunocanalicular organization. Enlarged dental pulp volume and predentin thickness in dKO and Hyp mice fed the high-phosphate diet indicated persistent dentin-pulp defects.

Conclusions: Genetic ablation of OPN in Hyp mice showed limited ability to improve dentoalveolar mineralization defects. OPN deletion dysregulates other mineralization-associated factors that should be investigated for their contribution to dentoalveolar pathology in XLH. Systemic phosphate correction partially improved mineralization defects independent of OPN.
Impaired Incisal Attachment and Eruption Following Bone Sialoprotein Conditional Ablation

Alexis N. Powers, Natalie L. Andras, Michael B. Chavez, Michelle H. Tan, Tamara N. Kolli, Brian L. Foster

Objectives: Bone sialoprotein (Ibsp gene; BSP protein) is an extracellular matrix protein found in the skeleton and dentition. Function(s) of BSP in mineralized tissue development remain unclear. In mice, global ablation of BSP results in impaired acellular cementum formation on the lingual root analogue of the continuously erupting incisor, detachment and disorganization of incisal PDL fibers, and increased rates of malocclusion. The reduced rate of incisor eruption in mice lacking BSP was shown directly by measurement of tooth movement and indirectly by more rapid closure of the incisor dental pulp. We hypothesized that BSP from the ectomesenchyme-derived dental follicle contributes to incisor attachment and eruption.

Methods: We developed a mouse carrying a floxed Ibsp allele (Ibspfl/fl) and conditionally deleted Ibsp from neural-crest-derived ectomesenchyme using Wnt1-Cre2 mice. Mandibles were harvested from Wnt1-Cre2+; Ibspfl/fl (conditional knockout – cKO) and wild-type (WT; Ibspfl/fl) control mice (n=5-6/group/time point) at 30 and 90-days-post-natal (dpn). Incisor attachment and eruption were analyzed by micro-computed tomography (micro-CT), immunohistochemistry, and histology.

Results: We used histology to assess the presence of cementum and PDL attachment. Immunostaining for BSP revealed its presence within the acellular cementum of WT mice and absence in cKOs. Compared to WT, cKO mice possessed absent or reduced acellular cementum, which led to PDL fiber disorganization and detachment. To evaluate the rate of incisor eruption, we measured incisal pulp volume by micro-CT. Analyses revealed incisal pulp volume was undiminished in cKO vs WT mice at 30 dpn. However, at 90 dpn, pulp volume decreased 90% (p<0.001) in cKO vs WT mice.

Conclusions: Conditional ablation of BSP from neural crest cells contributes to lack of cementum, structural defects at the cementum-PDL interface, and reduced eruption. These results mimic the incisor-associated periodontal breakdown in mice where BSP is globally ablated and highlight the importance of BSP in maintaining periodontal health and function.

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A Narrative Review in Periodontitis Patients Suffering from Cardiovascular Diseases

Bishoy Sadek, Binnaz Leblebicioglu

Background and Objective: Bacteremia due to severe forms of periodontitis and its effect on atherosclerosis is well documented with in vivo and clinical studies. It is also known that severe periodontitis may induce systemic inflammation which is a key player in coronary heart diseases. This narrative review aims to investigate current evidence on a possible association between cardiovascular and periodontal diseases in a microbiological, immunological and genetic scope.

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

Results: Periodontal bacteria can be detected in atherosclerotic tissues. Many common inflammatory markers such as TNF-α, IL-1β, IL-6, and C-reactive protein observed in patients with periodontal disease may also be observed in patients with cardiovascular disease. Moreover, environmental factors including but not limited to smoking, obesity, diabetes mellitus, hyperlipidemia, and socioeconomic status have been shown to correlate with both periodontitis and cardiovascular disease. Several candidate genes including ANRIL loci variants may induce predisposition to periodontal and cardiovascular diseases in some populations.

Conclusion: The causative evidence still does not exist between periodontal and cardiovascular diseases. However, there are strong evidence by common microbiological, immunological and genetic factors supporting co-existence of severe forms of periodontitis and cardiovascular diseases compared to periodontally healthy individuals.
SSRI Effect on Cranial Base Development in a Mouse Model

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Objectives: The prevalence of antidepressants in the United States, including selective serotonin reuptake inhibitors (SSRIs), has increased in the recent decade from 10.6% to 13.8%. Commonly, patients in the adolescent age bracket and pregnant women are prescribed these medications for a variety of psychosocial issues. The effects of SSRIs and other antidepressants on developing structures of the human body are largely unknown. Since SSRIs have been noted to affect cellular processes in bone and cartilage development, the cranial base synchondroses may be at risk. This study aims to elucidate the possible effects that SSRIs have on this development and subsequent formation of craniofacial features.

Materials and Methods: Wild type C57BL6 mice were used to produce litters that were exposed in utero to an SSRI, Citalopram (500 µg/day). μCT images were obtained on P15 skulls using a SkyScan 1176 (Bruker Kartuizerseg 3B, 2550 Kontich, Belgium) scanner. Scans were collected on 50 animals. Murine cranium μCT images from P15 pups were analyzed using AnalyzePro software to assess changes in cephalometrics and cranial base synchondroses.

Results: Cephalometric analysis indicated an overall morphological change with shortened cranium length in the exposed group compared to control (P = 0.013). The sphen-occipital synchondrosis (SOS) showed morphological changes in width, height, and AP length with width and height shortened in the exposed group and AP length expanded in the exposed group (P = 0.003, P = 0.016, P = 0.009 respectively). The intersphenoidal synchondrosis (ISS) showed changes in width and AP length with width shortened in the exposed group and AP length expanded in the exposed group (P = 0.017, P < 0.001 respectively).

Conclusion: The results of this analysis indicate an effect of SSRI exposure on the development of the cranial base and thereby developing structures of the calvaria and murine face. These findings are imperative in the fields of orthodontics and oral and maxillofacial surgery as SSRI exposed patients may require intervention.
Mechanical Stability of Bone Decreases with High Phosphate Diet

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Objectives: High consumption of processed foods can increase serum levels of inorganic phosphate (Pi). While many studies showed that the high Pi may cause chronic kidney disease, adverse cardiovascular complications, and skeletal muscle dysfunction, a lack of knowledge exists for its effects on bone health. Thus, the objective of this study is to examine whether a high phosphate diet can alter mechanical stability of teeth and femur in high and normal phosphate diets of a mouse model.

Methods: Following IACUC approval, 20 C57BL6N mice (20- to 24-week-old male) were fed a normal phosphate (NP; n=10) or a high phosphate (HP; n=10) with 0.9% and 2.3% total Pi for 12 weeks, respectively. Four mandibles (2 for each group) and 18 femurs (9 for each group) were randomly dissected from animals and subjected to static loading and dynamic mechanical analysis (DMA). A non-destructive compressive static displacement was applied to teeth and femurs up to 0.01 mm followed by cyclic loading (-0.01 ± 0.005 mm 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. A t-test was used to compare the NP and HP groups. Significance was set at 0.05.

Results: Stiffness (139.84±24.42 N/mm) and energy dissipation (0.049±0.004) of the HP group were significantly lower than those (196.44±35.18 N/mm and 0.055±0.008) of the NP group (p ≤ 0.05).

Conclusion: The current findings indicate that a high Pi diet likely decreases mechanical stability of bone. Future studies using the increased number of jaw bones are underway.
Bacterial metabolism of JUUL e-cigarettes adversely impacts oral Health

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Objectives: We have previously demonstrated that JUUL e-cigarettes adversely impact the host response to pathogens. However, their impact on the oral microbial-mucosal interactions is still unknown. Since the oral microbiome plays an essential role in maintaining health we investigated the effects of JUUL on host-microbial interactions using a combined multi-omics approach.

Methods: A customized Microbial Mucosal Interface Construct (MiMIC) was created with six pioneer species (commensal interface), followed by Fusobacterium nucleatum (secondary interface), and eight tertiary colonizers (tertiary interface) overlaid on EpiGingival organoid tissue. MiMICs were exposed to air or “Virginia Tobacco” flavor JUUL in 3% or 5% nicotine. Tissue and biofilm mRNA were sequenced and annotated to PANTHER and KEGG databases, respectively. Total-ion-mobility spectroscopy time-of-flight (TIMS-TOF) was performed to identify bacterial metabolites. Targeted inflammatory mediators were measured using multiplexed bead-based flow cytometry. Supervised multivariate analysis was performed using mixOmics for R.

Results: Multi-dimensional analyses revealed that JUUL metabolites clustered by both bacterial composition and nicotine concentration. Key metabolites common to all JUUL samples were flutamide (antiandrogen), valdecoxib (anti-inflammatory), and nizatidine (antihistamine). The metatranscriptome demonstrated varying patterns of bacterial gene expression driven by the type of JUUL. Two-way -omics analyses of host transcriptome and metabolome demonstrated that metabolic byproducts differentially impact host and microbial transcription, the key metabolites being sucrose, N-acetyllactosamine (glycoprotein), and omagripin (dipeptidyl peptidase-4 inhibitor). Key genes that were up- or downregulated in response to these metabolites included transcriptional repressor GATAD2B, hexokinase HKDC1, ubiquitin-protein ligase RNF144A, calcium homeostasis modulator CALHM2, divIva, gltX, bgsB, and recD, which contribute to cell division, protein biosynthesis, glycerolipid metabolism, and DNA replication/repair pathways.

Conclusions: Composition of the oral microbiome is a key determinant of JUUL metabolism and the downstream impact on host-microbial interactions. JUUL e-cigarettes can alter host-microbial interactions independent of the nicotine content.
Structural and Histomorphological Assessment of ADAM10 Conditional-Knockout Mouse Enamel

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Objectives: Due to its high mineral content and hierarchal crystal arrangement, dental enamel is the hardest tissue in the body. Many proteins and proteinases expressed by ameloblasts have been characterized for their role in enamel formation; however, the roles of several proteins remain understudied. Of note, a Disintegrin And Metalloproteinase Domain-10 (ADAM10), a transmembrane endopeptidase expressed in early stages of enamel development, may have a functional role in enamel formation. We characterized the structural and histological phenotype of enamel in the Adam10 conditional-knockout (cKO) mouse.

Methods: Mice carrying a floxed Adam10 allele (Adam10<sup>fl/fl</sup>) were bred with Amelx-iCre mice to conditionally ablate Adam10 from ameloblasts (cKO; Amelx-iCre-Adam10<sup>fl/fl</sup>). The physical and surface characteristics of enamel were assessed by micro-computed tomography (µCT), scanning electron microscopy (SEM), and nanoindentation on 7-week old wild-type (WT; Adam10<sup>fl/fl</sup>) and cKO mice. Hematoxylin and eosin (H&E) staining was performed on 5- and 12-days-post-natal incisors and maxillary first molars.

Results: µCT analysis revealed a delay in initiation of enamel mineralization and decreased enamel volume in cKO compared to WT mice. SEM results showed abnormal loss of a decussating pattern of enamel rods. Mechanical testing revealed cKOs possessed reduced enamel hardness and elasticity. Day-5 molars exhibited disorganization and detachment of ameloblasts from the enamel matrix. In contrast, at day-12, ameloblast morphology was unaltered. In cKOs, H&E of the developing incisor revealed retention of enamel matrix proteins, as indicated by the eosin stained layer persistent up until the incisal tip.

Conclusions: Loss of ADAM10 in ameloblasts results in compromised enamel quality and quantity. These results provide strong evidence for a role for ADAM10 in developing enamel.
Evidence for Novel Sexual Dimorphism in Jaw-adductor Protein Expression

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Objective: It is broadly recognized that human rheumatoid arthritis (RA) results in severe inflammation and peripheral joint pain. How RA impacts the masticatory system has not been well studied. The objective of this project was to examine and compare limb and jaw-adductor muscle protein expression patterns in a collagen-induced mouse model of RA to assess how RA impacts skeletal muscle.

Methods: The mice (13 control, 12 RA, both sexes) were euthanized and muscles were isolated, homogenized, and prepared for analysis with SDS-PAGE. The expression of two proteins appeared, from visual inspection of the stained gels, to be increased in the extensor digitorum longus muscle (molecular weight ~30 kDa) and masseter (~60 kDa) in the RA mice, compared to control mice. The relative amounts of both proteins, relative to a selected standard protein in the same samples, were quantitated using ImageJ.

Results: Statistical significance of the differences between the mean values of both proteins was determined with the paired t-test. Neither protein was significantly upregulated in the RA group (P values: P30 protein, 0.069; P60 protein, 0.088). However, P60 was expressed at a significantly (P = 0.002) higher level in the masseter of male, compared to female, mice, independent of RA status.

Conclusion: It is well documented that there are sex-related differences in contractile protein expression in skeletal, especially craniofacial, muscles in rodents. However, none of the proteins known to be expressed differently between male and female rodent jaw adductors has a molecular weight of ~60 kDa. The results add to the extent of sexual dimorphism in protein expression in rodent jaw-adductors.
Conditional Ablation of Bone Sialoprotein Impairs Cellular Cementum Formation

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Objectives: Bone sialoprotein (Ibsp gene; BSP protein) is an extracellular matrix protein in bones and teeth, including acellular (AC) and cellular (CC) cementum. Global ablation of BSP impairs AC formation, leading to periodontal breakdown. Effects of BSP ablation on CC remain unclear; reduced quantities of CC in young, global-knockout mice appear to catch up to controls at older ages. To test the tissue-specific functions of BSP, we conditionally deleted Ibsp from ectomesenchymally-derived tissues and analyzed: 1.) developmental effects on CC mineralization and 2.) CC apposition following a challenge model of unopposed supra-eruption. We hypothesized conditional-knockout mice possess reduced CC apposition following supra-eruption.

Methods: To analyze dentoalveolar development, mandibles were harvested from wild-type (WT; Ibspfl/fl) and conditional-knockout (cKO; Wnt1-Cre2;Ibspfl/fl) mice (n=6/group/timepoint) at 30 and 90 days-post-natal. To analyze the effect of supra-eruption on CC formation, maxillary first molars were bilaterally extracted at 6-weeks-of-age, and first mandibular molars were analyzed at 21-days-post-procedure (dpp) (n=3-4/group/timepoint). Tissues were analyzed by histological staining (hematoxylin and eosin) and immunohistochemistry (BSP and osteopontin (OPN)).

Results: Immunostaining revealed the presence of BSP in WT and absence in BspWnt-cKO CC. At 30 dpn, BspWnt-cKO first molars possessed reduced CC (40%;p<0.01) and increased CC cementoid. At 90 dpn, the difference in CC was less pronounced (24%;p<0.05). Prior to supra-eruption, BspWnt-cKOs possessed slightly less CC compared to WT. However, following supra-eruption, CC area was approximately equal between WT and BspWnt-cKOs. Deposition of OPN, a mineralization inhibitor, was unaffected in BspWnt-cKO CC.

Conclusions: Conditional ablation of BSP contributed to impaired CC formation, which normalized with increasing age. BSP was not essential for CC apposition during supra-eruption. These results suggest BSP has different developmental influences on AC vs CC.

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SEMA7a Localization is Disrupted in Tgfbr2 Conditional Knockout Dental Pulp

Monica Stanwick and Sarah B. Peters

Objectives: Innervation is a vital component for the accurate use and protection of our dentition. Our lab developed a conditional knockout mouse model with Tgfbr2 deleted in the mesenchymal pulp cells (Tgfbr2^cko) that demonstrated reduced mineralization in bones and teeth during postnatal development. Interestingly, we also found sparse and disrupted dental pulp (DP) innervation despite the fact that Tgfbr2 was not deleted in neuronal populations. We performed RNA Sequencing Analysis (RNA-Seq) on postnatal day 7 (P7) DP from control and Tgfbr2^cko mice and identified a panel of semaphorins and their receptors as potential mediators of innervation disruption, including Neuropilin 1 (Nrp-1), Plexin A2 (Plxna2), Plxna4, Plxnd1, Semaphorin 3a (Sema3a), Sema3f, Sema4a, Sema4b, and Sema7a. Because SEMA7a is an axonal chemoattractant and its RNA expression was downregulated in the Tgfbr2^cko mice, we hypothesized that Tgfbr2 regulates dental pulp innervation via downstream Sema7a.

Methods: We performed immunofluorescence experiments on developing Tgfbr2^cko and WT teeth to label SEMA7a. Fluorescent microscopy was used to detect SEMA7a expression levels and localization.

Results: We found SEMA7a localized to the odontoblasts in the DP of WT mice, similar to that previously reported for human teeth. However, SEMA7a was not present in the Tgfbr2^cko odontoblasts, but was instead localized to the DP tissue bordering the odontoblast layer.

Conclusions: Deletion of Tgfbr2 resulted in aberrant localization of SEMA7a correlating with decreased neurite outgrowth in developing teeth, suggesting the process of tooth innervation requires SEMA7a to attract and promote neurite outgrowth during postnatal development. Future experiments will examine developing teeth in Sema7a null and conditional knockout mice to determine whether tooth innervation is disrupted in a manner similar to Tgfbr2^cko mice. Defining the mechanisms governing tooth innervation and mesenchymal-neuronal crosstalk will enable the development of better strategies to restore, regenerate, and maintain healthy teeth.
Artificial intelligence for predicting the Index for Orthodontic Treatment Need

Leah Stetzel, Tai-Hsien Wu, Henry Fields, Fernandas Schumacher and Ching-Chang Ko

Introduction: One of the most widely used assessments of orthodontic treatment need is the Index of Orthodontic Treatment Need (IOTN). Multiple studies have verified the reliability and validity of the IOTN. The IOTN-Aesthetic Component (AC) defines esthetic impairment into ten levels; Level 1 represents the least treatment need, while Level 10 represents great need. However, the grading of IOTN is subjective. In this project, we propose the use of artificial intelligence (AI) to augment IOTN assessment which would allow for objective diagnoses, a reduced workload for orthodontists, and at-home assessments of orthodontic treatment need.

Objectives: The specific aim of this study is to collect a dataset of patients’ oral images with the corresponding IOTN-AC classification and propose a deep-learning based algorithm that can assess oral images and identify the need for orthodontic treatment.

Methods: Pre-treatment frontal intraoral photos were collected and graded by a gold-standard rater. Intra-rater reliability was assessed. AI was trained using the verified intraoral images and two different schemes (called Model 1 and Model 2). The training data was annotated as 1 to 10 (representing IOTN-AC Level) in Model 1 and as 1 to 3 (representing the “No need, borderline need, and great need”) in Model 2.

Results: Our gold-standard rater had intra-rater reliability using weighted kappa of 0.84 (95% CI 0.76-0.93). Model 1 had an average of 62% sensitivity, 79% specificity, and 68% accuracy. Model 1 had positive predictive value (PPV) of 74% and a negative predictive value (NPV) of 83%. Model 2 had an average of 53% sensitivity, 78% specificity, and 67% accuracy. Model 2 had PPV of 82% and a NPV of 84%.

Conclusion: We have developed an AI system that can automatically predict treatment need based on IOTN-AC reference standards. Results can presumably be improved with an increase in sample and training size.
Distance Dependent Changes of Mechanical Properties at Bone-implant Interface

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Objective: Active bone remodeling at the bone-implant interface following an implantation surgery alters the interface bone properties. A histological analysis showed that new bone tissues are formed up to 300 µm from the surface of the implant. Mechanical properties of the newly formed interfacial bone tissue is important in determining the primary stability of an implant system. Thus, the objective of this study was to examine whether mechanical properties of the interface bone tissues change with distances from the surface of the implant.

Materials and Methods: Histological slices of the bone-implant interface were made at 3 months after implantation in a canine mandibular model used for a previous study. The histological specimens were subjected to nanoindentation in the current study. Using the load/displacement curves for a cycle of indentation, hardness (H), viscosity (η) and viscoelastic creep (Creep/f), and elastic modulus (E), were obtained at peak load, holding, and unloading processes, respectively. Viscoelastic tangent delta (tan δ) was assessed using oscillatory phase angle (δ) shifts during holding. A total of 110 indentations were performed at the distances between 30 µm to 600 µm from the surface of the implant. The nanoindentation parameters were compared between within and farther than 300 µm using a t-test and their variations with distances were examined using Pearson's correlations with a significance level of p<0.05.

Results: The E values were statistically significantly greater at farther than 300 µm from the implant (p=0.01) significantly increasing with distances (p=0.008). All other parameters were not significantly different with distances (p>0.05).

Conclusion: The E values increase with higher mineralization of bone tissue. As such, the current findings indicate that mineralization of bone tissue develops progressively with distances from the surface of the implant, which likely plays an important role in maintaining mechanical stability of the implant system.
Oral Streptococci Interactions with Each Other and Their Environment

Emily Williams and Justin R. Kaspar

Objectives: Oral streptococci within dental plaque communities can be associated with health or disease. In the past, the caries-causing pathogen *Streptococcus mutans* has been studied primarily by itself (i.e., in monoculture). 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 upregulated when *S. mutans* was cocultured with either *Streptococcus gordonii* or *Streptococcus sanguinis*. Our hypothesis is that these identified upregulated genes are important for the competitive fitness of *S. mutans* when growing in the presence of health-associated oral streptococci. Additionally, we compared the growth of *S. mutans* within cocultures in human saliva versus regular lab-based growth medium (TYG).

Methods: Twelve different mutant genes, including the parental strain, 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 kanamycin and spectinomycin agar plates in order to enumerate colony forming units (CFUs) of *S. mutans* and the commensal oral streptococci mixture separately. Growth of the cocultures in human saliva and TYG were analyzed using a BioTek Synergy H1 Microplate Reader.

Results: We identified two out of eleven genes screened 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 mutant cocultures. *S. mutans* within cocultures in human saliva showed a significant increase in growth compared to TYG.

Conclusions: Through our competitive fitness assay, we have identified two specific *S. mutans* genes that result in decreased fitness during coculture after their loss. This study emphasizes the importance of studying *S. mutans* in cocultures rather than in monoculture. The next step for this project is to evaluate why *S. mutans* grows differently in human saliva compared to TYG. By gaining a broader understanding of coculture competition and the environment in which they grow, we hope to develop new therapeutic strategies to prevent dental caries in the future.
Organization of epithelial proteins in the Ddr1 Knockout Mouse Model

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Objectives: The oral epithelium functions as a first-line defense against microbial attacks on the oral cavity. Discoidin Domain Receptor 1 (DDR1) is a transmembrane, non-integrin collagen receptor highly expressed at the epithelial basal layer. Mice not expressing the receptor (Ddr1−/−) show defects consistent with periodontitis such as epithelial down growth and alveolar bone loss. We aimed to characterize and compare the spatiotemporal expression of oral epithelial proteins, specifically E-cadherin and collagens IV, VIII, and XVII in the Ddr1−/− mouse model. We hypothesized that the absence of DDR1 would affect epithelial protein organization.

Methods: Immunohistochemistry (IHC) was used to identify the localization of E-cadherin and collagens IV, VIII, and XVII (important components for epithelial cell junctions or epithelium-connective tissue attachment) in Ddr1+/+ and Ddr1−/− mice at 30 and 270 days post-natal (n=4-5 mice/genotype/time point).

Results: In this study, we investigated E-cadherin due to the reported close association of this marker and DDR1 in epithelial adherens junctions, collagen XVII because it is an important component of hemidesmosomes attaching epithelial cells to the basement membrane, and collagen VIII due to its role in the attachment of the epithelium to the tooth surface. Collagen IV was also investigated as it is a major ligand of DDR1 and a key component of the basement membrane attaching epithelium to the underlying connective tissue. IHC did not reveal differences in the localization of E-cadherin or collagens IV, VIII, and XVII between Ddr1+/+ and Ddr1−/− mice at any of the ages of development.

Conclusions: Our study showed no significant effect of DDR1 on the organization of E-cadherin and collagens IV, VIII, and XVII. Further studies are needed to identify the mechanism behind the effect of DDR1 on the epithelial component of periodontal tissues.
Permeability of Junctional Epithelium in Ddr1 Knockout Mice

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Objectives: Epithelium is the periodontal first line of defense against microbial attacks. The permeable junctional epithelium (JE) works as a selective barrier controlling passage of microbes, gingivocrevicular fluid, antimicrobial substances and immune cells. Discoidin Domain Receptor 1 (DDR1) is a transmembrane non-integrin collagen receptor significantly expressed at the basal epithelial layer. Ddr1−/− mice develop defects consistent with periodontitis, including JE downgrowth, bacterial invasion and alveolar bone loss. Thus, the objectives of this study were to examine the JE permeability in absence of DDR1. We hypothesized that Ddr1−/− mice would exhibit increased JE permeability, resulting in elevated microbial influx in the deep periodontal tissues and periodontal destruction.

Methods: Epithelial permeability was studied in vivo by applying Dextran-Texas Red, a fluorescent dye, in the oral cavity (close to the gingival sulcus of maxillary molars) of Ddr1+/+ and Ddr1−/− mice (n=8/genotype) at 10 weeks of age. Surface area and intensity of dye permeating through the JE was measured. Student t-test was used for statistical analysis.

Results: Interestingly, but opposite to what we were expecting, there was a strong trend for Ddr1−/− mice to exhibit decreased JE permeability compared to Ddr1+/+ mice, as expressed by decreased dye intensity (p=0.12) and dye surface area (p=0.09) in the deep periodontal tissues of the former mice.

Conclusion: Our data indicate that Ddr1+/+ mice have decreased JE permeability than Ddr1−/− mice. Additional studies will be performed to confirm and extend these findings.
Extraoral Storage Time: Effect on Autologous Gingival Graft Early Healing

James L. Zaiger and Dimitris N. Tatakis

**Objectives:** Gingival grafting is a predictable method to increase keratinized tissue and treat gingival recession, but reestablishment of vascularization is a vital step for graft integration at the recipient site. Current clinical consensus is that recipient beds should be prepared first to minimize the length of time the graft is kept extraorally to reduce risk of graft necrosis. However, there is no clinical evidence that supports this practice. The aim of this prospective split-mouth experimental clinical trial is to assess how extraoral gingival graft storage time affects graft healing and revascularization.

**Methods:** Adult, systemically healthy, non-smokers were recruited. 6mm diameter and 1.5mm thick gingival grafts were removed from both sides of the palate. Surgery was timed so that each recipient bed remained open for 20 minutes, with the test (T) graft kept in room-temperature saline for 40 minutes and the control (C) graft kept in saline for 2 minutes before being placed at the contralateral harvest bed and fixed with two drops of cyanoacrylate. Clinical photos, intraoral scans, and gingival blood perfusion, measured via laser doppler flowmetry (LDF), were taken pre-operatively, post-operatively, and at each follow-up visit. Healing Score Index (HSI) was assessed at mesial graft margin on post-operative days 2 (D2), 3 (D3), 7 (D7), and 14 (D14).

**Results:** Preliminary results are reported from 6 patients who completed all study visits. No graft from either group underwent necrosis or failed to heal by D14. Average(±sd) time recipient beds remained open was 21.2±1.5 min. Average extraoral storage time was 1.5±0.5 min and 42.3±2.9 min for C and T group, respectively (p<0.0001). Postoperative graft perfusion (LDF) values peaked on D7 for both groups. Compared to immediate post-implantation, graft perfusion recovery was not different between groups, at all postoperative time points (p>0.458). HSI on D2, D3, D7, and D14 did not differ significantly between groups (p>0.22) and all C and T grafts achieved the maximum possible HSI score on D14.

**Conclusion:** Within the limitations of these preliminary results, extraoral storage time for up to 40 minutes has no significant detrimental effect on autologous graft early healing and revascularization. Supported by the OSU Division of Periodontology.