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Poster Presentation

#1. Angiopoietin-like 4 increases resistance of HNSCC to cisplatin through enhanced DNA damage response and HR-mediated DNA damage repair

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Head and neck squamous cell carcinoma (HNSCC) poses a significant clinical challenge, with a stagnant 5-year survival rate of approximately 50% despite considerable treatment efforts. Current therapeutic approaches involve surgical resection followed by radiation and/or cisplatin-based chemotherapy. Unfortunately, cisplatin resistance rapidly emerges, contributing to unfavorable outcomes in advanced HNSCC cases. Addressing this challenge is imperative for enhancing treatment options and overcoming resistance.

Our lab studies Angiopoietin-like 4 (ANGPTL4), a pro-angiogenic factor associated with diverse aspects of cancer progression such proliferation, migration, invasion, anoikis resistance, metabolism, and angiogenesis. Previous work in our lab has revealed heightened expression of ANGPTL4 in HNSCC cells and patient tissues. Our investigations establish a pivotal role for ANGPTL4 in HNSCC cell migration. For our present study, we have hypothesized that ANGPTL4 is involved in promoting DNA repair and augmenting HNSCC resistance to cisplatin via its impact on ABL1 activity.

Our findings demonstrate that elevated ANGPTL4 expression diminishes HNSCC cell sensitivity to cisplatin, mitigates DNA damage induced by cisplatin treatment, and enhances the efficacy of repair processes, as evidenced by extrachromosomal homologous recombination assays. Moreover, ANGPTL4 significantly elevates RAD51 phosphorylation at Y315 and Y54, events linked to enhanced RAD51 invasion and strand exchange activity in homologous recombination repair. Phosphomutant studies confirm the necessity of increased RAD51 phosphorylation for ANGPTL4-mediated enhancement of homologous recombination repair. Notably, our investigation implicates neuropilin 1 (NRP1) and ABL1 in this pathway.

Ongoing research delves into the molecular mechanisms regulating ANGPTL4's impact on homologous recombination repair and cisplatin resistance. We are also exploring the therapeutic potential of NRP1 and ABL1 inhibition in overcoming cisplatin resistance in HNSCC. This project aims to unravel the intricate mechanisms underpinning ANGPTL4-dependent cisplatin resistance, providing valuable insights into the therapeutic targeting of this multifaceted protein as a novel strategy for treating HNSCC.

#2. Unraveling the Role of eSTK Signaling and PBP4 in Antibiotic Resistance of *Staphylococcus aureus*

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Staphylococcus aureus typically resides as a commensal within the human microbiota, but has the ability to become an opportunistic pathogen, causing various infections in both community-associated and hospital-acquired settings. Treatment remains challenging due to the emergence of highly resistant strains such as MRSA (Methicillin-Resistant *Staphylococcus aureus*). Classically, β-lactam resistance in *S. aureus* has been attributed to β-lactamase production (encoded by *blaZ*) or Penicillin-Binding Protein 2a (PBP2a, encoded by *mecA*). While the mechanism of action of PBP2a is well-studied, a lot about the regulation of *mecA* remains unknown. Through growth assays, real-time PCR and immunoblotting, we demonstrate that Stk1 and Stp1, a two-component ekaryotic-like serine/threonine kinase system that regulate biological and metabolic functions in bacteria, play an essential role in regulating *mecA* expression. Deletion or inhibition of *Stk1* rendered bacteria susceptible to β-lactams, highlighting its pivotal role in *mecA* mediated resistance.

PBP4 (Penicillin Binding Protein-4), a protein involved in cell wall synthesis and maintenance, has been identified as a non-classical mediator of resistance. Mutations detected in the regulatory site of *pbp4 (Ppbp4*)* led to increased protein expression and enhanced cell wall crosslinking, resulting in resistance to β-lactams. We explored the effects of increased cell wall crosslinking and demonstrated that it led to resistance to other classes of antibiotics as seen by a reduction in survivability in growth assays and caused a decrease in virulence in the animal model *Caenorhabditis elegans*. When present alongside a functionally altered or inactivated GdpP, PBP4-associated mutations resulted in significant increase in minimum inhibitory concentration (MIC) for β-lactams, suggesting a synergistic role in resistance.

Our studies with both, classical and non-classical mechanisms of resistance in *S. aureus* provide insights into the mode of action of key players involved in each of these mechanisms and reveal their potential as therapeutic targets for combating antibiotic resistance.

#3. Pathological claustrum activity drives aberrant cognitive network processing in human chronic pain

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Aberrant cognitive network activity and cognitive deficits are established features of chronic pain. However, the nature of cognitive network alterations associated with chronic pain and their underlying mechanisms require elucidation. Here, we report that the claustrum, a subcortical nucleus implicated in cognitive network modulation, is activated by acute painful stimulation in healthy participants. Moreover, we discover pathological activity of the claustrum and a region near the posterior inferior frontal sulcus of the right dorsolateral prefrontal cortex (piDLPFC) in migraine patients during acute pain and cognitive task performance. Migraine patients exhibit increased activation compared to controls in both regions in both tasks. In healthy participants, piDLPFC activity is observed only during painful stimulation, but migraine patients recruit this region during painful stimulation and pain-free cognitive task processing. Dynamic causal modeling suggests a directional influence of the claustrum on piDLPFC activity, and diffusion weighted imaging verifies their structural connectivity. These findings advance understanding of claustrum function during acute pain and provide evidence of a possible circuit mechanism driving cognitive impairments in chronic pain.

#4. EFFECTS OF GREEN LIGHT THERAPY ON PAIN-LIKE BEHAVIOR AND INFLAMMATORY MARKERS IN AN OSTEOARTHRITIS MODEL IN RATS

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Knee osteoarthritis (OA) is a leading cause of chronic pain disproportionately affecting women. Proinflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), and interleukin 6 (IL-6) are key effectors of knee OA pathogenesis, inducing painful joint inflammation and cartilage degeneration. Despite standard knee OA interventions, patients still report pain. Green light has emerged as a potential therapeutic for various pain conditions, with hypoalgesic effects reported in fibromyalgia and chronic migraine patients and in animal models of chronic pain. However, the effects of green light-emitting diode (GLED) exposure on an inflammatory condition like knee OA are understudied, especially considering sex. Here, we induced knee OA in male and female Sprague Dawley rats via intra-articular knee injection of monoiodoacetate (MIA, 3mg/15µL). To assess primary hyperalgesia, knee mechanical thresholds were measured using a small animal algometer (smalgo, Bioseb). Two days post-injection, rats were exposed to GLED or ambient room light (ARL) eight hours daily for 24 days. Every three days up to 24 days post-injection, mechanical thresholds were recorded, with a final measure taken five days after light exposure termination (30 days post-injection). Blood serum levels of TNF-α, IL-1β, and IL-6 were quantified at baseline and 23 days post-injection using an ELISA. GLED- and ARL-exposed rats exhibited a marked decrease in mechanical threshold one day post-injection. GLED exposure attenuated mechanical hyperalgesia in both sexes compared to ARL controls—an effect that was maintained five days after light exposure termination. GLED-induced hypoalgesia occurred sooner (six GLED sessions) and with greater magnitude in males. GLED exposure decreased blood serum proinflammatory cytokine levels. These results show that GLED exposure attenuates mechanical hyperalgesia in the MIA model, which can be partially explained by reduced circulating proinflammatory cytokines. Sex differences appeared in the treatment-matched groups, which may reflect sex differences in the mechanisms of OA pain maintenance and recovery.

#5. 3D PRINTING POLYCAPROLACTONE-ALLOGRAFT BONE CONSTRUCTS WITH SLOW RELEASING OSTEOINDUCTIVE NANOGELS

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Critical-sized bone defects (CSBDs) affect 1 in 20 people globally. Bone allografts are commonly used during CSBD procedures but lack essential growth factors for mature bone formation. Also, it remains challenging to contour allograft into complex CSBD void geometries. The objective of this work is to 3D-print polycaprolactone (PCL)-allograft constructs coated with bone morphogenetic protein-2 (BMP-2)-loaded nanogels (BMP-nanogel) to regenerate bone in CSBDs. A 3D extrusion printing strategy was employed to fabricate 3-6 mm-diameter constructs made of PCL and 0, 10, or 30 wt% human bone allograft with 250, 500, or 750 µm interconnected pores. BMP-2 was loaded into nanogels composed of poly(N-isopropylacrylamide)-dextran-poly(lactate-2-hydroxyethyl-methacrylate) by UV-emulsion polymerization. Survival of dental pulp stem cells (DPSCs) with the constructs and nanogels was evaluated by MTT assay. Nanogel localization and adherence on the constructs were assessed by fluorescence. BMP-2 released from nanogels was quantified by ELISA and BMP-2 bioactivity was assessed by BRE-luc reporter assay. The bone regenerative capacity of the constructs, with and without BMP-nanogel, was evaluated in both mice heterotopic ossification and rat critical sized calvarial defect models by histology and micro-CT 50 days post implantation. Incorporation of 30 wt% allograft in PCL constructs increased nanogel binding affinity to the construct, and DPSC proliferation by 50% after a week in culture. Nanogels were not cytotoxic to DPSCs at concentrations up to 5 mg·mL-1. Nanogels released BMP-2 with near zero-order release kinetics for 35 days. BRE-luc assay revealed that the BMP-2 in the nanogels had nearly equal bioactivity as control BMP-2. Histology and micro-CT showed that allograft and BMP-2-loaded nanogels played important roles in increasing construct cellular penetration and mineralization in rodent models. The 3D-printed BMP-2-nanogel-containing constructs, made of hybrid PCL and allograft, have great potential to provide 3D-scaffold and slow releasing osteoinductive factors for patient-specific and defect-site-specific bone regeneration.

#6. Thermoresponsive and Biodegradable Nanogel Systems for RNA based Therapeutics

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RNA based therapeutics have attracted significant interest for gene editing, vaccines and immunotherapy. The main bottleneck for gene therapy is the effective delivery of genetic material. Nanocarriers show great promise for transporting drugs across biological barriers, reducing drug clearance, and improving the bioavailability of drugs at the target. Polymer-based nanocarriers have evolved as promising systems for the delivery of various therapeutics due to their tunability and robustness. Hydrophobicity and surface charge of nanoparticles are two of the main factors that affect the size, composition and shape that affect permeation through biological membranes and drug release. The objective of this work is to show the robustness and the tunability of polymeric systems to control the size, permeability, cellular uptake and siRNA release for gene therapy. Thermoresponsive and biodegradable nanogels composed of dextran-hydroxyethylmethacrylate with polycaprolactone (DEX-PCL-HEMA) and N-isopropylacrylamide (NIPAAM) monomer were synthesized using Irgacure® 2959 as an UV initiator. To render nanogels negatively, positively or not charged, 0, 2, 5 and 10 mol% of acrylic acid (AA) or 2 amino-ethyl methacrylate (AM) was added with respect to NIPAAM. Cy3-labeled siRNA was added to the mixture following proper procedure to prevent degradation. The size of the nanogels got smaller with increase in charge due to increase in hydrophilicity. Nanogels were not toxic to ARPE-19 cells up to concentration of 2 mg/mL. The negatively charged nanogels were taken up mostly by 6 hours, while the positively charged nanogels took 1 day. In the span of 4 hours, nanogels permeated across the cellular membrane significantly higher than the control. Our system was able to sustain release the siRNA for 7 months. The thermoresponsive and biodegradable nanogels showed no sign of toxicity and the surface charges played an important role in the size, permeability, cellular uptake of nanogels and the release duration of nucleotides.

#7. Characterization of the Oral Microbiome in a COVID-19 Patient Cohort

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Objectives: The oral cavity remains an underappreciated site for SARS-CoV-2 infection despite the myriad oral conditions observed in COVID-19 patients. However, recently, SARS-CoV-2 was shown to replicate in salivary gland cells resulting in inflammation. Given the established association between oral inflammation and microbiome disruption, we aimed to comparatively profile oral microbial shifts at a metagenomic level in a prospective study using a cohort of COVID-19 patients hospitalized at the University of Maryland, Baltimore Medical Center.

Methods: Oral swabs were obtained from all enrolled COVID-19 patients (n=26) and healthy control subjects (n=30) for DNA sequencing. Additionally, swabs were also taken from the oral cavity for fungal culturing to evaluate colonization by the opportunistic fungal pathogen *Candida albicans*, the etiologic agent of oral candidiasis. To identify potential COVID-19 associated pathologic dysbiotic shifts in the oral microbiome, we performed comprehensive shotgun metagenomic sequencing on all samples.

Results: Comparative analysis indicated that overall, COVID-19 patients exhibited significantly reduced bacterial and viral diversity/richness (alpha diversity). We identified 12 differentially abundant bacterial species, all of which were negatively associated with COVID-19. Incidental abundances of fungal species were observed in COVID-19 patient samples and upon culturing, the majority of COVID-19 samples were positive for *C. albicans*; in contrast, no *Candida* was recovered from any of the control samples.

Conclusions: We expect on going functional metagenomic analysis to illuminate the role played by COVID-19 in metabolic community dynamics of the oral microbiome, which could potentially allow for the emergence of putative pathogens. With the current lack of emphasis on implications of COVID-19 on oral health, these findings may provide lacking insights that may lead to reassessment of risks for susceptibility for development of oral candidiasis during COVID-19 disease.

#8. Development of a Novel Calcium Phosphate Cement as a Pulp Capping Material

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Objectives: While Mineral Trioxide Aggregate (MTA) is considered a gold standard for pulp capping, challenges such as prolonged setting times and high costs persist. Metformin, a commonly prescribed primary medication for managing type 2 diabetes, has the potential to induce osteodifferentiation and promote mineralization. This study aimed to (1) develop a calcium phosphate cement (CPC) with enhanced physio-mechanical characteristics, aiming to overcome MTA's drawbacks, and (2) Explore the impact of CPC-metformin on the attachment and proliferation of human dental pulp stem cells (hDPSCs), and measure metformin release.

Methods: The CPC powder comprised a 1:1 molar ratio mixture of tetracalcium phosphate and dicalcium phosphate anhydrous. The liquid chitosan malate was formed by dissolving chitosan in a 60% mass concentration of malic acid, then lyophilized for future use. CPC specimens, prepared at varying powder-to-liquid ratios by mass (2:1, 2.5:1, 3:1, 3.25:1, 3.5:1, and 4:1), underwent evaluation for physio-mechanical properties in comparison to MTA (ProRoot MTA, Dentsply Sirona, Tulsa OK). The materials were assessed for flexural strength, elastic modulus, work-of-fracture, flowability, and setting time. The optimized CPC ratio was then loaded with 0, 50, 100, and 150 μg of metformin to measure metformin release over 28 days and assess hDPSCs attachment and proliferation at 24 hours.

Results: CPC at a 3.25:1 ratio successfully reduced the cement setting time to (41.5 ± 2.1 min) as compared to (123 ± 4.2 min) for MTA ($p < 0.05$), while matching the other mechanical and physical properties of MTA. The amount of released metformin corresponds proportionally to its concentration, with CPC-150 at 203.20 μg/mL, CPC-100 at 115 μg/mL, CPC-50 at 83.37 μg/mL, and CPC-Zero at 0.85 μg/mL. Live/dead assay illustrated that both CPC formulations, with or without metformin, exhibited excellent cell viability (>92%) and facilitated cell attachment similar to MTA.

Conclusion: CPC demonstrated comparable physio-mechanical properties at a 3.25:1 powder-to-liquid ratio, with a significantly shorter setting time than MTA. The hDPSCs adherence and excellent viability were unaffected by CPC-metformin, compared to conditions without metformin or with MTA. Preliminary data suggests that the novel CPC-metformin, with its shorter setting time and cost-effectiveness, holds promise for pulp capping, but further investigation is required.

#9. Detection of Intracranial and Extracranial Carotid Calcifications in Cone Beam Computed Tomography Utilizing a Deep Learning Convolutional Neural Network Image Segmentation Approach

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Introduction: Atherosclerosis is a major risk factor for myocardial infarction and stroke. Carotid calcifications (CC), a reliable indicator of future myocardial infarction and stroke, can incidentally be detected on head and neck cone beam computed tomography (CBCT) during routine dental visits. CBCT can identify and quantify calcifications as small as $1mm^3$ and even mild calcifications are proven to be associated with significant coronary artery disease. CBCTs are now widely used in dentistry for surgical planning and diagnosis with over 5.2 million CBCTs exposed every year in the USA. However, it is estimated that only 29% of x-rays are reviewed by an appropriate specialist and therefore the potential for undiagnosed incidental cardiovascular pathology is high.

Materials and Methods: This study aimed to leverage an artificial intelligence (AI) deep learning convolutional neural network image segmentation approach to detect incidental CC on CBCT images. Transfer learning *via* a U-Net based neural network architecture was utilized. A total of 137 axial CBCT images were included and distributed as 60% training, 10% validation, and 30% testing.

Results: Mean training and validation accuracy for extracranial image segmentation was 92% and 82%, respectively. Pixel testing accuracy for extracranial CC was 92%, with an area under the curve (AUC) of 0.84, a sensitivity of 100%, and a specificity of 69%. Intracranial CC detection had a sensitivity of 93%, AUC of 0.5, and a specificity of 8%.

Conclusion: The deep learning model showed excellent sensitivity for the detection of extracranial and intracranial CC. The findings of this study highlight the potential to utilize AI methods for medical image analysis. The findings also demonstrate how AI can alert or flag the clinician to discovery of serious incidental pathology in the oral and maxillofacial region and the potential to enhance early detection of future incident cardiovascular complications.

#10. Development of a Novel Resin-Based Antibacterial Root Surface Coating Material

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Abstract: Root caries caused by cariogenic bacteria presents a burden on a large number of individuals worldwide, especially the elderly. As people are living longer and keeping their natural teeth into old ages, the prevalence of root caries is continuously increasing. Coating exposed root surfaces has the potential to inhibit caries initiation and progression, thereby protecting the natural teeth. The objectives of this study were to develop a novel antibacterial coating to inhibit root caries and to investigate the antibacterial efficacy of the resin-based coating with antibacterial monomer dimethylaminohexadecyl methacrylate (DMAHDM).

Methods: DMAHDM was synthesized via a modified Menschutkin reaction. A resin was formulated using 55.8% urethane dimethacrylate (UDMA) and 44.2% of ether-based triethylene glycol divinylbenzyl ether (TEG-DVBE) (all mass %) at filler:matrix mass ratio of 10:90. Silanized barium boroaluminosilicate glass particles (Dentsply Sirona, Milford, DE, USA) was used as a filler. The antibacterial monomer was incorporated at different mass fractions (0%,3%,5%, and 7%). The mechanical and physical properties were assessed via flexural strength, modulus of elasticity, flow test, and degree of conversion. Antibacterial properties were tested with 48-hour *Streptococcus mutans* (*S. mutans*) biofilms grown on resin disks. The colony-forming units (CFUs), metabolic activity, and lactic acid production by biofilms were assessed.

Results: The coating with or without DMAHDM exhibited a higher flexural strength than that of a commercial control Seal & Protect (Dentsply DeTrey GmbH, Konstanz, Germany) ($p < 0.05$). The modulus of elasticity and flow test results were similar to commercial control ($p > 0.05$). The experimental groups, particularly those with 5% and 7% DMAHDM, demonstrated degree conversion that was not significantly different from that of the commercial control. The incorporation of 3% DMAHDM resulted in a 3-log reduction in *S. mutans* biofilm CFU when compared to commercial control ($p < 0.01$), while the 5% and 7% DMAHDM groups showed an 8-log reduction. The use of DMAHDM resulted in a significant reduction in both biofilm biomass and lactic acid production ($p < 0.01$).

Conclusion: The incorporation of DMAHDM into a novel coating resin showed excellent physical and mechanical properties compared as well as a potent antibacterial reduction against *S. mutans* biofilms. This bioactive coating is promising to protect the exposed roots against sensitivity,

abrasion and caries in patients undergoing crown lengthening procedures, periodontal surgeries, or the elderly with gingival recession.

#11. Impact of Root Canal Disinfection on Endodontic Microbiome

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Objectives: To investigate endodontic microbiome in primary infection with apical periodontitis (PIAP) and assess the impact of root canal disinfection on endodontic microbiome.

Methods: This clinical study included twenty-four patients with PIAP. Samples were collected before disinfection (s1), after disinfection with 2.5% NaOCl (s2), and after supplemental disinfection with XP-endo Finisher file (s3). A quantitative polymerase chain reaction (qPCR) was performed to compare the bacterial load between different sampling timepoints (s1, s2 and s3). Illumina MiSeQ V3-V4 sequencing of 16s rRNA gene amplicons was performed, and sequence data were processed using the DADA2 pipeline, with taxonomy assignment against the Human Oral Microbiome Database (HOMD). Alpha diversity was determined using Shannon, Simpson, ACE, Chao1, and Fisher indexes. Beta diversities were determined. Beta diversity analysis was performed, and Principal coordinate analysis of Bray Curtis distances was plotted. Core microbiome and differential abundance analyses were performed using Analysis of Composition of Microbiomes (ANCOM).

Results: There was a significant reduction in bacterial load from s1 to s2 ($P<.05$). The supplemental disinfection (s3) failed to improve the root canal disinfection (s2) ($P>.05$). Alpha diversity was lower in s1 than s2 and s3 ($p<.05$). s1 exhibited clustering, and PERMANOVA showed a significantly distinct community type from s2 and s3, exhibiting a more heterogeneous composition and differentially abundant taxa. The root canal disinfection significantly impacted the relative abundance by decreasing Bacteroidetes, Fusobacteria, and Synergistetes and increasing Firmicutes and Proteobacteria at s2 and s3.

Conclusions: Microbiome in PIAP is complex and has high microbial heterogeneity among the patients. The root canal disinfection reduced the bacterial load and impacted the endodontic microbiome, revealing distinct community features. The long-term relevance and determinants of the residual microbiome ground the development of a more targeted future root canal therapy.

#12. Silencing of nociceptive afferents attenuates hyperalgesia and marginally prevents condylar degeneration of TMJ after injury.

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A common cause of nondental pain in the orofacial region is from temporomandibular disorder (TMD). One of the painful conditions of TMD is temporomandibular joint osteoarthritis (TMJOA). It causes slow degeneration of subchondral bone and deteriorating of its cartilage, which often is accompanied by pain. However, the mechanistic association of nociceptive afferentsand TMJ degeneration is not clearly established. Sixty five percent of trigeminal ganglia afferents projected to TMJ contains calcitonin gene-related peptides. Approximately half of which co-expresses transient receptor potential vanilloid 1 (TRPV1), which likely mediates pain from TMJ. To determine the contribution of nociceptors to TMJ degeneration and hyperalgesia, we functionally manipulated the TRPV1-lineage afferents in a noninvasive way. We used an inhibitory designer receptor exclusively activated by designer drugs (DREADD), an engineered receptor coupled with inhibitory G protein which can silence targeted neurons upon binding to clozapine-N-oxide (CNO), a specific activator. For targeting the expression of hM4Di, we used $TRPV1^{Cre}$ mice. We used forced mouth opening (FMO) as a model for TMJ injury, leading to TMJ degeneration and hyperalgesia. To activate hM4Di, CNO-loaded Alzet osmotic pump was implanted in the back of the animal before starting the FMO procedure, which allows for chronic release for 7 days. We preformed micro-computed tomography (μCT) to assess subchondral bone phenotypes in mandibular condyles. Silencing of TRPV1-lineage afferents attenuated spontaneous pain assessed by mouse grimace scale, whereas TMJ degeneration was only modestly affected. Our results suggest that TRPV1 afferent fibers may not be a primary contributor in condylar degeneration following TMJ injury.

#13. Effect of Polydopamine-RGD Coating of PMMA on Gingival Fibroblast Attachment

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Objectives: Creating an optimal peri-implant mucosal seal around dental implant abutments is essential for ensuring long-term implant stability. Surface modifications, particularly the addition of texture, play a significant role in facilitating the early healing of soft tissues. However, it is important to note that such modified surfaces tend to be prone to bacterial colonization. Previous study demonstrated that polydopamine-mediated surface modification with the cell adhesion peptide RGD enhanced cell attachment on titanium implant abutments, without altering the surface roughness. Nonetheless, further research is required to ascertain whether this polydopamine-mediated RGD coating approach is applicable to other commonly used abutment materials, such as poly(methyl methacrylate) (PMMA). In the current investigation, we aimed to assess the effectiveness of polydopamine-mediated RGD-functionalized PMMA surfaces in promoting human gingival fibroblast early attachment.

Material and Methods: Surfaces were prepared on milled PMMA discs consisting of 4 groups: (1) unmodified; (2) polydopamine (PD)-coated; (3) PD-RGD-coated; (4) PD-RGE-coated. Functionalization was confirmed via contact angle and surface roughness prior to cell seeding. Human gingival fibroblasts at 3X10/cm were seeded to examine the early attachment (2 hours) via a Cell Counting Kit-8. One-way ANOVA was used for statistical analysis.

Results:
1.There was no difference in surface roughness before and after the coating of the materials. 2.Polydopamine had a significant effect in decreasing the contact angle, thus increasing the wettability ($p < 0.05$).3. Early attachment data showed the PD-RGD-coated surface resulted in significantly higher fibroblast cell attachment than other groups.

Conclusions: Our data supports the idea that polydopamine-facilitated RGD surface modification can serve as a valuable strategy to enhance the biocompatibility of PMMA as implant abutment material. Further studies are needed to evaluate the clinical application of this approach.

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#14. Health Disparity in Early Childhood Caries: Unveiling the Potential Ethnicity-specific Microbiome

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The profound disparity in tooth decay among young children (early childhood caries) has been demonstrated in national surveillance data for many years, particularly regarding race/ethnicity and poverty level. While various factors, such as family, culture, health insurance, and infrastructure have been explored, the biomedical perspective specific to the population remains underexplored. Employing RNASeq technology, our cross-sectional study examines the functional characteristics of plaque bacteria microbiomes and their transition from non-disease to disease status. Dental plaque samples were collected from caries lesions and non-caries lesions (paired) in same individual from 19 minority children with ECC and 12 healthy subjects with similar age, ethnicities, and socioeconomic background for baseline establishment. Across all subjects, 7793 genes significantly changed in caries status, with 4970 unique to African American with ECC and 6519 to Latin American Hispanic children with ECC. Common ECC-related bacteria like *Streptococcus mutans* and *Lactobacillus rhamnosus*, alongside lesser-known species, *Veillanella parvula* and *Propionibacterium acidifaciens*, contributed to these changes. Notably, African American children with ECC exhibited shifts linked to *Pseudopropionibacterium propionicum* and *Cardiobacterium hominis*, while Hispanic ECC children showed contributions from *Propionibacterium acidifaciens*, *Selenomonas sp.*, *Rothia dentocariosa*, *Atopobium parvulum*, and *Streptococcus sanguinis*. Metabolic functions of the genes significantly changed in caries plaque varied among all subjects and between ethnic groups. This study highlights diverse metabolic pathways in plaque bacteria contributing to ECC in minority populations, identifying bacterial species beyond common cariogenic bacteria. This information could translate to the development of personalized risk assessment and interventions thus delivering more efficient and cost-effective outcomes.

#15. Spatial lipidomics of SARS-CoV-2 infected mouse lungs

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COVID-19 disease is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus. Pulmonary infection with SARS-CoV-2 can result in significant lung inflammation, triggering tissue destruction and loss of function. The constant mutations and significant variants, with variable clinical significance, both changing the disease manifestation and impacting vaccine efficacy. Alterations in phospholipids (PL) have been related to lethal inflammatory response in mouse infection models and, using a mass spectrometry imaging (MSI) approach, it is possible to map differential concentration and spatial distribution of this PLs in infected lung tissue. We sought to understand the PL alterations in mouse lungs infected with SARS-CoV-2 to identify interventional targets in lipid metabolism. The mice were intranasally infected with PBS (mock control group) and with 10^5 PFU of SARS-CoV-2 B.1.351. Triplicate lung tissues were collected after 2- or 4-days post-infection with mock infected lungs collected at 4 days. The lungs were inflated with gelatin and stored at -80°C prior to sectioning and analysis. Lungs sections of 10µm thickness were mounted on glass slides, Norharmane matrix was applied and, for the MSI analysis, a timsTOF Flex was used in positive and negative ion modes with 20µm spatial resolution. The control group showed a higher intensity of several PL classes than the infected groups suggesting a strong divergence of the phospholipid profile between the groups. In infected lungs, the spatial distribution of several PLs changed, concentrating in organized spots within the infected groups compared to the control group with no correlation to obvious histological features representing an apparent molecular fingerprint. Follow up studies are underway to determine the cellular and subcellular features that can account for this unique spatial PL fingerprint. There is a rearrangement in the spatial distribution of PLs linked to the time of infection and these alterations might be related to immune modulation caused by virus replication or wound repair mechanisms.

#16. The Role of Gut Microbiota in Skeletal Homeostasis

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Objective: Growing evidence indicates that perturbations in gut microbiota can lead to skeletal disorders such as osteoporosis and rheumatoid arthritis, however, the underlying mechanisms remain poorly studied. The aim of this proposal is to elucidate the causal role of dysbiotic gut microbiota in skeletal disorders using unique pre-clinical models.

Methods: Global and intestinal-specific interferon regulatory factor 8 (Irf8) knockout (KO) mice mirroring human gut dysbiosis and osteoporosis conditions were compared against wild-type (WT) mice with commensal gut microbiota. To discern the direct effects of gut microbiota, these mice were generated under germ-free conditions and respective gut microbiota were inoculated into them. Microbial profiling was performed using shotgun metagenomics sequencing, and skeletal phenotyping was performed using micro-CT, histology, cell cultures, RT-qPCR, W.B, ELISA, etc.

Results: Commensal gut microbiota in WT mice affected intestinal, systemic, and skeletal health. However, dysbiotic gut microbiota in Irf8 KO mice exacerbated these effects, causing marked intestinal and systemic inflammation, immune cell alterations, changes in bone metabolism markers, increased OC formation, and reduced bone mass. The intestinal inflammation in Irf8 KO mice was characterized by increased fecal LCN2 levels and a higher Th17/Treg cell ratio, which was accompanied by increased intestinal permeability. Systemic inflammation was evidenced by elevated circulatory levels of LPS, IL17, and TNFα. Furthermore, the impaired bone health in Irf8 KO mice was evidenced by decreased in-vivo trabecular bone volume, trabecular number, and trabecular thickness and increased in-vitro OC numbers, resorption activity, and OC-related genes/proteins. Collectively, these results suggest that dysbiotic gut microbiota causes intestinal inflammation, compromised gut barrier permeability, and altered immune cell and cytokine expression, which in turn lead to enhanced osteoclastogenesis and reduced bone mass.

Conclusion: This preliminary study offers novel insights into the causative role of gut microbiota in skeletal disorders, highlighting potential strategies for modulating gut dysbiosis to improve skeletal health.

#17. Characterization of structurally engineered lipopoligosaccharide and its potential immunomodulatory role in Alzheimer's Disease.

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Structurally engineered variants of lipopolysaccharide (LPS) are effective tools for the manipulation of the innate immune response. Key challenges remain, though, including cumbersome extraction methods, the efficacy of ectopically expressed lipid A modifying enzymes, and the possibility of using engineered live bacterial strains to therapeutically colonize the gut. Here, we developed a first-generation strain of *E. coli* addressing several of these key challenges. Using a lipid-A structural engineering tool called bacterial enzymatic combinatorial chemistry (BECC), we engineered an *E. coli* variant, D31m4 bearing the dual lipid A modifications: 3-O-deacylation and 1-dephosphorylation (Ec DUAL). The resulting product is monophosphoryl lipid A (MPLA)-like lipooligosaccharide (LOS). Structural characterization of Ec DUAL LOS was carried out via MALDI-TOF MS using the novel rapid extraction method, termed fast lipid analysis technique (FLAT). Variants of lipid A can control inflammatory activity through a finely tuned structure-activity relationship with Toll-like Receptor 4 (TLR4). TLR4 structure-activity property of Ec DUAL LOS was tested in human TLR4-expressing HEK293-Blue cells which showed a weak, competitive agonist-like activity of Ec DUAL LOS. TLR4 is consistently implicated in Alzheimer's Disease (AD) etiology. A recent study has shown that chronic stimulation of the TLR4 pathway by a partial agonist (MPLA) significantly improved AD-related pathology in AD mouse model. The goal of the current study was to use inexpensive BECC method to effectively produce TLR4-modulating lipid A structures and to characterize their structure-activity relationship and correlate those with mechanistic outcomes in an AD mouse model. Furthermore, we also studied the dissemination of the parenterally administered product to the brain which showed dissemination by 2 hours and persisted for at least 12 hours. In a mouse gut repopulation study, the Ec DUAL live strain was found to temporarily colonize the gut and could be positively selected to improve the duration of colonization. Studies are ongoing to understand the impact of chronic treatment with Ec DUAL LOS in various AD mouse models.

#18. STRESS IN RATS INCREASES THE SUSCEPTIBILITY TO MIGRAINE-LIKE ACTIVATION AND SENSITIZATION OF TRIGEMINOCERVICAL NEURONS: INFLUENCE OF SEX

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Migraine headache is a severe and highly disabling pain condition that affects females more than males. It is thought to be mediated by abnormal activation and sensitization of intracranial meningeal afferent neurons and their central projection to the trigeminocervical complex. Chronic stress affects millions of people worldwide, and it is established that patients with migraine are particularly susceptible to stress as a migraine trigger, although the exact mechanism is unknown. The aim of the study was to dissect the role of stress and possible sex dimorphism in reducing the threshold to mediate these migraine-like mechanisms. We adapted and combined two migraine-like approaches, stress and a chemical migraine provocative (nitric oxide (NO) donor), to dissect clinically relevant neurophysiological changes within trigeminovascular neurons. Rats (male and female) were stressed using either restraint (2h for four days) or food-fasting (16h). This was followed by administration of a non-noxious dose of the NO donor, sodium nitroprusside (SNP; 30µg/kg, IV). *In vivo* electrophysiological extracellular recording of dural-responsive trigeminocervical neurons was carried out in naïve or stressed rats with responses evaluated post-SNP injection. Migraine-like periorbital sensitivity was measured using von Frey filaments. Only in naive females did SNP mediate activation and sensitization of dural-responsive trigeminocervical Aδ and C-fiber neurons, where the activation threshold of intracranial meningeal afferents was also significantly lower. In the stressed/SNP rats, significant neuronal changes were observed in both sexes. However, responses were significantly exacerbated in stressed females compared to naïve. Female rats showed a lower periorbital withdrawal threshold and delayed recovery from stress than males. These data demonstrate that stress can independently mediate migraine-like periorbital hypersensitivity, indicative of sensitization of dural-trigeminocervical neurons. Both stresses also ‘prime’ the trigeminovascular system, making it more susceptible to migraine provocatives (NO donors), translating to the increased susceptibility of migraine patients to stress. We also demonstrated clear sex differences in the sensitivity and activation of intracranial meningeal afferents that translate to the experience in the clinical setting.

#19. DISRUPTION OF MITOCHONDRIAL PYRUVATE OXIDATION IN DORSAL ROOT GANGLIA DRIVES PERSISTENT NOCICEPTIVE SENSITIZATION AND CAUSES PERVASIVE TRANSCRIPTOMIC ALTERATIONS

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Metabolism is inextricably linked to every aspect of cellular function and defects in metabolic pathways are associated with a variety of neoplastic, cardiovascular, and neurodegenerative diseases. Additionally, polymorphisms and inborn errors in metabolic genes are associated with a multitude of chronic pain conditions. Nerve growth factor (NGF) is another factor that associated with a multitude of chronic pain conditions, but its potential role in regulating the metabolism of sensory neurons is largely unknown. Hence, we explored whether intraplantar NGF injection in mice alters the metabolism of sensory neurons. We determined that intraplantar NGF injection disrupts mitochondrial pyruvate oxidation leading to an increased extrusion of lactate and protons. These changes were driven by the enhanced expression of pyruvate dehydrogenase kinase 1 (PDHK1) and lactate dehydrogenase A (LDHA). Moreover, pharmacological, and genetic blockade of PDHK1 and LDHA attenuated NGF-induced tactile allodynia. Direct disruption of mitochondrial pyruvate oxidation in dorsal root ganglia was sufficient to cause tactile allodynia that lasted at least 3 months - unlike NGF-induced allodynia which resolves within 72 hours. Full-length transcript analysis revealed that the disruption of mitochondrial pyruvate oxidation significantly altered the transcription of large number of genes, reduced the poly-A tail length of mRNAs, and modified the alternative splicing of hundreds of transcripts. These findings demonstrate that aberrant mitochondrial pyruvate oxidation is a crucial mechanism for the development and maintenance of chronic pain.

#20. METFORMIN AND OTHER METABOLIC INHIBITORS ATTENUATE NEUROPATHIC PAIN AND TUMOR GROWTH IN MICE WITH PARANEOPLASTIC SYNDROME AND CIPN

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Chemotherapy-induced peripheral neuropathy (CIPN) and paraneoplastic neurological syndrome are two conditions that can cause significant pain and discomfort in cancer patients. CIPN is a common side effect of certain chemotherapeutics and can result in numbness, tingling, and pain. Paraneoplastic neurological syndrome, on the other hand, is a rare disorder that occurs when cancer-fighting antibodies attack parts of the nervous system. Both neuropathies can persist which can adversely affect the quality of life and the rehabilitation of cancer patients. Unfortunately, therapies that can alleviate tumor or chemotherapy-induced neuropathic pain that do not interfere with tumor growth do not currently exist. The main goal of this study was to identify a therapeutic strategy that can achieve both anti-tumor and analgesic effects. The chemotherapeutic, bortezomib, has been shown to induce aerobic glycolysis in sensory neurons which lead to bortezomib-induce neuropathic pain. Aerobic glycolysis is also a hallmark of cancer cells, suggesting a common metabolic vulnerability. Paraneoplastic neuropathies are commonly associated with lung cancers. Hence, we used Lewis Lung Carcinoma cells (LLC1) to develop a mouse model of paraneoplastic neuropathy. We hypothesized that blocking metabolic pathways could alleviate CIPN and paraneoplastic neuropathic pain without compromising on tumor control. To test our hypothesis, we demonstrated that mice implanted with LLC1 developed significant allodynia. Treatment with bortezomib attenuated tumor growth but exacerbated the neuropathic pain. However, co-treatment with metformin, which blocks bortezomib-induced aerobic glycolysis in sensory neurons and prevents CIPN, attenuated both tumor growth and neuropathic pain. Similarly, inhibition of lactate dehydrogenase and pyruvate dehydrogenase kinase with oxamate and dichloroacetate respectively, also reduced tumor growth and pain. These results suggest that targeting metabolic pathways is a promising strategy to improve oncologic outcomes and alleviate neuropathic pain in cancer patients.

#21. Transcriptional Signatures and Functional Analysis of *Candida auris In Vivo* Grown Biofilms Reveal Functional Redundancy in Cell Wall Adhesins Crucial for Cell-Cell Interaction and Coaggregation

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Candida auris is an emerging pathogen associated with life-threatening invasive disease due to high level of transmissibility and multi-drug resistance. *C. auris* strains display aggregative and non-aggregative growth phenotypes with different biofilm forming abilities. In this study, we aimed to identify unique transcriptional signatures associated with the aggregative phenotype during biofilm growth. We performed comprehensive RNA-Seq analysis on cells of both phenotypes following *in vitro* grown biofilms and on biofilms recovered from infected catheters implanted subcutaneously in mice. Mutant strains of upregulated genes were generated using CRISPR-Cas9 and phenotypic evaluations were performed using confocal laser scanning microscopy and scanning electron microscopy for biofilm formation, and single-cell force spectroscopy to measure cell-cell adhesion forces. Comparative analysis identified a set of genes consistently differentially upregulated in the aggregative strain under *in vitro* and *in vivo* conditions with key roles in adhesion and biofilm formation. Two genes exhibiting highest upregulation were identified as *ALS5* and a gene since named *SCF1* with partial similarity to the *C. albicans* Rbt1 cell wall adhesin involved in cell-cell adhesion and coaggregation. We generated mutant strains of each gene in the aggregative strain; phenotypic evaluations demonstrated significantly reduced biofilm formation for the ***Δscf1*** mutant but not for the ***Δals5*** compared to the wild-type strain. Although cells of mutants of each gene lost their coaggregation capability when tested individually, when mixed, aggregation was comparable to that of wild-type strain. Cell-cell affinities were also demonstrated by measuring adhesion and rupture forces using single-cell force spectroscopy. Our findings demonstrated significant transcriptional changes associated with the aggregative form with cell wall adhesins that although with little similarity, may have complementary roles and function redundantly to promote cell-cell interaction and biofilm formation. Functional diversity of cell wall proteins may be a form of regulation providing the *C. auris* aggregative phenotype with flexibility and rapid adaptation to the environment.

#22. The Impact of Titanium Surface Modifications on Gingival Cell Growth.

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Objectives: With osseointegration becoming the “minimum standard” for implant survivability, research focus has turned to soft tissue interface at the dental implant and/or dental implant abutment and the surrounding soft tissue for improved long-term success. With the increased occurrence of peri-implantitis being reported, there have been arduous efforts made to improve soft tissue compatibility. In this study, we investigated four different modifications on Titanium that are well-accepted materials in clinic and evaluated their impact on gingiva fibroblast growth.
Material and Methods: To test this, our experimental model for this study included human gingival fibroblasts (HGF), which represents part of the peri-implant soft tissue, as well as four different types of materials—Disc A (Osseotite CP Gr4), Disc B (Machine + NTs Alloy) Disc C (As-Machine CP), Disc D (As-machine alloy), see Figure 1. Cell growth was monitored using cell Counting Kit-8 assay in order to test different of soft tissues healing.
Results: Among all the discs, Disc C and D (no significant difference between C and D) favored more cell growth compared with Disc A and B. The data collected by the end of 168 hours growth showed Disc B, C, D had similar cell growth patterns. However, Disc A revealed less cells on its surface compared with the other discs. A growth curve was plotted, which showed all materials supported HGF growth; however, Disc A has a slower growth rate compared with other discs. *$P<0.01$
Conclusions: From this limited data, Disc B, C, and D may be a potential superior alternative for abutment material than Disc A. More studies including other soft tissue cell types, such as human gingival keratinocytes are necessary to determine the most optimal modification of soft tissue biocompatibility.

#23. COMPARISON OF VIRTUAL TREATMENT SETUP AMONG CLEAR ALIGNER COMPANIES

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Over the past two decades, clear aligner therapy grew as an alternative to fixed braces for orthodontic treatment. Recently, several orthodontic companies introduced their clear aligner brands, each with unique features such as material type and thickness, gingival trim design, specific auxiliary tools, and different strategies for guiding orthodontic movement. However, little is known about how these features impact their virtual treatment setup.

The aim of this study was to compare the virtual treatment setups among five clear aligner companies to assess their differences. The initial records of 10 patients, including extra and intraoral photos and scans, were submitted to Invisalign®, Clear Correct®, 3M™ Clarity™, Spark™, and Reveal™ clear aligner systems. Each case prescription was standardized to ensure comparable treatment plans across the companies.

The comparison focused on the number of aligners, number of attachments, amount of interproximal reduction per arch, planned extrusive or intrusive movement of maxillary central incisors, final canine and molar relationships, final intercanine and intermolar widths, and planned expansion or constriction of the intercanine and intermolar widths.

Results indicated significant differences in the virtual treatment setups for the same patient among clear aligner companies in the number of aligners (p-value = 0.003), number of attachments (p-value < 0.001) and predicted final canine relationship (p-value = 0.013). However, no statistical differences were observed in the other variables evaluated.

ClearCorrect® stands out by prescribing the fewest number of aligners and attachments, whereas 3M™ Clarity™ tends to prescribe the highest number of aligners and attachments. There is a notable deficiency across companies, particularly with ClearCorrect® and Spark™, in planning for a final bilateral canine Class I relationship.

These findings suggest that the unique characteristics of each aligner company lead to distinct approaches in treating the same patient, highlighting both areas of discrepancy and consistency in the virtual treatment setups.

#24. Factors affecting tooth loss among diabetic patients at an academic institution

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Purpose: While studies have reported that diabetic individuals are at greater risk for periodontal disease and tooth loss, few studies have examined whether periodontal disease is the primary factor affecting tooth loss in diabetic individuals. The purpose of this retrospective study was to investigate factors affecting tooth loss among diabetic individuals in comparison to nondiabetic ones.

Methods: 202 participants were included after screening 298 ones with extractions completed in 2022. The exclusion criteria were as follows; 1) only third molar extraction performed, 2) age ≥89, and 3) only remaining roots left. Patient- and tooth-related data were collected. Descriptive statistics were prepared for each independent variable such as age, gender, periodontal, smoking and diabetes status. The multiple linear regression (MLR) model was built to investigate the association between the number of remaining teeth and selected predictors.

Results: The study population includes 48 diabetic (24%) and 154 nondiabetic individuals. The diabetic group was significantly older, exhibited more periodontitis, and retained significantly fewer teeth compared to the nondiabetic group. 187 participants (90%) lost their teeth due to nonperiodontal reasons: there was no difference in the reasons for tooth loss between the two groups. When age and smoking were considered, diabetes did not significantly affect the odds of having periodontitis and did not significantly affect the number of remaining teeth.

Conclusions: Factors other than periodontal disease contributed more substantially to tooth loss in both groups. Age and smoking were significant factors affecting the remaining teeth number, regardless of periodontitis and diabetic status.

#25. Pediatric Feeding Therapists' Knowledge of Children's Oral Health

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Children with pediatric feeding disorder (PFD) experience greater barriers to dental care than healthy children. Some of these barriers are associated with oral health professionals, such as refusal to treat patients with special healthcare needs and lack of resources and education associated with treating patients with special healthcare needs. As a result, children with PFD need the support of non-dental healthcare professionals to aid in caring for their oral health. The goal of this study was to evaluate the oral health knowledge, confidence, and practices among healthcare providers who manage children with PFD. Two hundred and twenty-five surveys were emailed to members of the International Association of Pediatric Feeding and Swallowing organization. The survey consisted of 25 questions to assess oral health-related knowledge, confidence, and practices. Participants were grouped together based on their reported occupation into 1 of 3 groups: group A: occupational therapists, speech-language pathologists, and feeding therapists (n=28), group B: psychologists (n=6), and group C: physicians, nurse practitioners, and registered dietitians (n=17). Chi-square and Fischer exact tests were used to determine statistical significance ($P<.05$). Fifty-one individuals (23% response rate) participated. 50% or more of participants correctly answered 6 of the 12 questions evaluating oral health knowledge. Oral health knowledge among groups was not found to be statistically significant ($P>.05$). 50% or more of participants reported “not confident” in 2 of 6 questions evaluating oral health confidence and reported “often” in 2 of 6 questions evaluating oral health practices. Statistical significance ($P<.05$) was noted between groups in both oral health confidence and practice questions. The results indicate that healthcare providers require support from oral health professionals to optimize the oral health of children with PFD. Support may include increased targeted educational programming and development of enhanced referral pathways for dental providers.