1A

Mapping the Influence of *Pseudomonas aeruginosa* (*Pa*) Infection on Host Lipid Profiles Using a Multi-Factorial CF-like Mouse Model

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Pseudomonas aeruginosa (*Pa*) is responsible for chronic lung infections in individuals with cystic fibrosis (CF). CF is one of most prevalent genetic diseases in the United States and is the result of mutations of the chloride transporter, the CF transmembrane conductance regulator (CFTR). Approximately 70% of CF patients become infected with *Pa* by adulthood, contributing to recurrent infection cycles and mortality due to respiratory failure. Despite the emergence of highly effective modulator therapies (HEMT), there remains a critical need for enhanced treatment strategies that directly target dysregulated aspects of infection and inflammation. A lung phenocopy mouse model of CF lung disease was used to characterize the lipid response to infection, specifically, mice that overexpress the β-subunit of the epithelial sodium chloride channel (βENaC). Adult, female βENaC mice on a BALB/c background were either treated with CFTR-inh172 or mock treatment to recapitulate the CFTR KO in these animals followed by infection with either a mucoid *Pa* clinical isolate (CF1188) or a nonmucoid isolate (CF001). We identified a regulated lipid response within the lungs of wildtype mice infected with a laboratory-adapted strain of *Pa*. These changes corresponded with reported trends observed in human lipidomic analyses of bronchial alveolar lavage fluid from CF patients. These lipid modifications are predominantly driven by neutrophil-mediated lipid remodeling. Using mass spectrometry imaging (MSI), we observed spatially distinct lipid changes in the βENaC lungs associated with increased mucus production, dominant enrichment in phosphatidylglycerol (PG) lipids and other phospholipids (PLs). Spatial lipidomic data from *Pa*-infected βENaC lungs showed a shift to phosphatidylethanolamine (PE) PLs, likely owing to the contribution from infiltrating neutrophils. Ongoing research aims to elucidate the role of functional versus inhibited CFTR in modulating neutrophil infiltration and its consequent effects on lipid population alterations in response to infection.

2A

Effects of Green Light Exposure on Pain-like Behavior and Periaqueductal Gray Connectivity in a Rodent Model of Knee Osteoarthritis

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Functional changes in pain-processing brain regions have been observed across various chronic pain conditions, including persistent pain related to knee osteoarthritis (OA), which disproportionately affects women. A prior study from our lab revealed that green light exposure with green light-emitting diodes (GLED) reduces primary hyperalgesia in a sex-dependent manner in the monoiodoacetate (MIA) model of knee OA. Preclinical studies have demonstrated that green light analgesia can be partly attributed to modulation of the pain-processing neuraxis. However, no studies have investigated the effects of GLED exposure on brain functional connectivity (FC) in a model of chronic pain using both sexes. Here, we examined the effects of GLED exposure on pain-like behavior and periaqueductal gray connectivity (PAG) in the MIA model of knee OA using the static weight bearing test and functional MRI (fMRI), respectively. GLED exposure attenuated weight bearing asymmetry induced by unilateral injection of MIA (3mg/15µL) in the left knee joint compared to ambient room light (ARL) exposure, with analgesic effects occurring sooner in males. fMRI data acquired 30-31 days after MIA injection revealed greater PAG FC with the rostral anterior cingulate cortex (rACC), insula, and primary somatosensory cortex in GLED-exposed rats. Additionally, greater PAG FC with the rACC, prelimbic cortex, motor cortices, and insula was observed in GLED-exposed males compared to their female counterparts. These results demonstrate that GLED elicits analgesia and alters PAG FC in a sex-dependent manner in the MIA model of knee OA. These findings warrant further investigation into the effects of GLED on PAG-based descending pain inhibition in both sexes.

3A

ANGPTL4-induced c-jun and STAT5 activation in the promotion of retinal neovascularization and vessel hyperpermeability in retinal endothelial in-vitro model

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Diabetic Retinopathy (DR), the most common microvascular complication in patients with diabetic eye disease (DED), is the leading cause of blindness among working-age adults. As a disease, its cause resides in the retina being exposed to an ischemic environment where angiogenic factors and hyper-permeability factors (HPFs) produce further damage to the retinal vasculature. Current treatments of DR based on targeting HPFs such as Vascular Endothelial Growth Factor (VEGF) fail to achieve clinically significant visual improvement in patients. Here, we studied the molecular basis and translational implications of the role of the novel DR biomarker, Angiopoietin-like 4 (ANGPTL4), and its receptor Neuropilin 1 (NRP1) in DR progression. ANGPTL4, as a cytokine, promotes NV (Neovascularization) and VHP (Vascular Hyper-Permeability) in ischemic models. Also, it is implicated in multiple pathological processes, such as cancer metastasis enhancing the EMT.

With these investigations, we were able to decipher early molecular signaling steps regulated by ANGPTL4. Using a global phosphorylation array in murine retinal endothelial cells (iRECs), we found a rapid activation of two transcriptional factors, c-jun and STAT5, associated in multiple cancer models with induction of angiogenesis and Vascular Hyper-Permeability (VHP) mechanisms. Further investigations using global cytokine arrays and protein profiling showed evidence of novel ANGPTL4-mediated mechanisms of ANGPTL4 action, that involve the secretion of Proliferin, a protein of the prolactin superfamily, and Angiopoietin 1. Functional assays showed that ANGPTL4 critically promotes angiogenesis and VHP in iRECs. Additional analysis with a soluble fragment of Neuropilin 1 (sNRP1) and an inhibitor of NRP1 shows a reduction in angiogenesis and VHP upon ANGPTL4 treatment in iRECs. Collectively, our investigations bring fundamental insight into the molecular mechanisms by which ANGPTL4 promotes DR and identify potential therapeutic targets that could be used as alternative molecular-based treatments for this devastating blinding disease.

4A

Eukaryotic-like serine/threonine kinase Stk1 mediates broad-spectrum β-lactam resistance in epidemic-causing strains of *Staphylococcus aureus* through attenuation of BlaI degradation

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Staphylococcus aureus is an important human pathogen that can cause life-threatening infections. *S. aureus* is famously resistant to many antibiotics, particularily to β-lactams, often leading to therapy failure. Classically, β-lactam resistance in *S. aureus* is mediated by two separate inducible pathways: β-lactamase (BlaZ) and Penicillin Binding Protein-2a (PBP2a) encoded by the genes *blaZ* and *mecA*. Their expression is mediated via their cognate repressors BlaI and MecI respectively. Activation of these pathways are controlled by sensory induction of the metalloprotease BalR1 and MecR1 respectively. Of the two resistance mechanisms, *mecA* produces broad-spectrum β-lactam resistance (also known as methicillin resistance).
In contemporary infection-causing, methicillin-resistant *S. aureus* strains such as USA300, sensory induction of *mecA* is controlled via the BlaR1-BlaI pathway. The eukaryotic-like serine/threonine kinase Stk1 has recently been shown to phosphorylate BlaR1. In this study, we demonstrate that Stk1 mediates a critical function in the production of broad-spectrum β-lactam resistance in contemporary infection-causing strains. In strains lacking a functional Stk1 (achieved via genetic manipulation and/or chemical inhibition), expression of BalZ and PBP2a is hampered, leading to broad-spectrum β-lactam susceptibility. Using qPCR and Western blotting we further demonstrate that Stk1 mediates efficient degradation of the BlaI repressor via BlaR1. Stk1 does not modulate the expression of the regulatory genes BlaR1 and BlaI, indicating that the mechanism of Stk1-mediated resistance occurs at the post-transcriptional level. Our findings in this study highlight the importance of Stk1 as a possible drug target as part of combination therapy with β-lactam antibiotics.

5A

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.

6A

Engineering Biologically Inspired Tissue Hybrids Towards Treating Patient And Defect-Site-Specific Bone Defects

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There are over 150 million new bone fractures globally each year. Critical sized bone defects (CSBDs) have minimal regenerative capabilities and are usually treated by bone grafts. However, bone grafts are either scarce or lack the essential growth factors and appropriate mechanical properties for mature bone formation. In our previous work, we invented 3D-print polycaprolactone-allograft (PCL-ALLO) constructs with interconnected pores ranging from 250-750 μm using a commercial fused deposition modeling (FDM) printer. Bone morphogenetic protein-2 (BMP-2)-loaded nanogels (BNGs) composed of poly(N-isopropylacrylamide)-dextran-poly(lactate-2-hydroxyethyl-methacrylate) were embedded in the constructs to aid bone healing in a heterotopic ossification mouse model. In this study, sonic hedgehog (SHH) was also loaded in the nanogels and the osteoinduction effects of BMP-2- and SHH- loaded nanogels on dental pulp stem cell (DPSCs) were evaluated via mineralization and alkaline phosphatase (ALP) assays. The effects of the 3D-printed constructs embedded with BMP-nanogels on bone healing were assessed in a rat critical sized calvarial defect model using micro-CT. The results show that the BMP-2 and SHH-nanogels were monodispersed to ~60 nm with up to 95% encapsulation efficiency. The nanogels sustained the release of biologically active BMP-2 and SHH for >7 weeks. The BMP-2-nanogels enhanced the osteodifferentiation of DPSCs and the SHH-loaded nanogels further augmented the osteodifferentiation of the BMP-2-nanogels. The 3D-printed constructs embedded with BMP-nanogels caused ~7% defect bone volume shrinkage after 2 months of implantation in the rats with critical sized calvarial defects. Completion of the project will have a significant impact on creating clinically translatable bone implants in combination of nanotherapy for patient-specific and defect-specific large scale bone regeneration.

7A

Biodegradable Nanogels for Controlled Release of Artemisinin-derived Drug to Treat Leukemia

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Acute myeloid leukemia (AML) is the most common acute leukemia in adults and children, with an average five-year survival rate of 30%, and much lower 11% for central nervous system (CNS) AML subtype. Despite recent advances in AML treatments, their limited efficacy and significant toxicity contribute to a high financial burden—estimated at $13.6B in 2020. Artemisinins (ARTs), with low or absent toxicity in their widespread use as malaria treatments, have shown activity against leukemia cells resistant to standard therapies. We developed a potent antileukemic ART analog ART631, yet its clinical use is hampered by poor water solubility and a short half-life. The objective of this project is to develop an ART631-loaded biodegradable nanogel system (NanoART631, PCT/US2023/019974 and US Patent Application 18/859,226) with enhanced water solubility and sustained release of ART631 to treat leukemia in both the blood and brain.

NanoART631 improved ART631's water solubility >400-fold. It had a z-average diameter of 100-200 nm, a low polydispersity index of<0.2, and a stable zeta-potential of -9 to -16 mV. NanoART631 sustained the release of ART631 over at least 35 days. *In vitro*, NanoART631 was taken up by three human AML cell lines and >98% of the cells were associated with NanoART631 within 2-hour incubation. NanoART631 had potent antileukemia activity against AML cells (IC_{50}<40nM), while being non-hemolytic(<2% at 80xIC_{50}) and non-toxic to two non-malignant cell lines. Additionally, NanoART631 permeated the *in vitro* hCMECs BBB model 1.5 times >4K dextran and 1.2 times >plain nanogels. *In vivo*, NanoART631 was well tolerated by NRG mice with a single-dose IV maximum tolerated dose(MTD) 500mg/kg (containing ~25mg/kg ART631), and empty nanoparticles had no clinical toxicity at concentrations ≥800mg/kg.

In conclusion, NanoART631 presents a novel antileukemic agent with significantly enhanced ART631 water solubility and sustained release to treat leukemia in both the blood and brain.

8A

ASCT2-mediated glutamine metabolism drives pain resolution

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Chronic pain is a debilitating condition with limited effective treatments. Understanding the mechanisms of pain resolution is crucial for developing novel therapies. This study uncovered a compensatory mechanism involving enhanced glutamine oxidation and upregulation of the glutamine transporter ASCT2 in the resolution of nerve growth factor (NGF)-mediated allodynia. Using the hyperalgesic priming model, we demonstrated that disruption of mitochondrial pyruvate oxidation persisted even after pain sensitivity returned to baseline. However, pain resolution was associated with increased glutamine utilization and ASCT2 expression in dorsal root ganglia (DRGs). Knockdown of ASCT2 prevented the resolution of NGF-induced allodynia and precipitated the transition to a chronic pain state. The glutamine catabolite dimethyl α-ketoglutarate (DKG) attenuated glycolytic flux and alleviated allodynia in both acute and chronic phases of the hyperalgesic priming model. Furthermore, ASCT2 knockdown prevented the resolution of allodynia in the plantar incision model. These findings highlight the critical role of adaptive metabolic responses in pain resolution and identify ASCT2-mediated glutamine metabolism as a potential therapeutic target for chronic pain. Understanding the endogenous mechanisms that promote pain resolution can guide the development of novel interventions to prevent the transition from acute to chronic pain.

Keywords: hyperalgesic priming, chronic pain, NGF, metabolism, ASCT2, DRG

9A

The effect of selective adenosine A3 receptor (A_3AR) agonism on migraine-like dural-responsive trigeminocervical neurons

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The adenosine A3 receptor (A_3AR) is a G_i protein-coupled receptor that is widely expressed in the central nervous system and could represent a safe, novel option for targeted therapy in pain disorders. Previous preclinical studies have shown that A_3AR agonism helps in alleviating neuropathic pain, however its role in trigeminally-related nociception and migraine is unknown. In this study, we aim to determine the effect of A_3AR agonists on migraine-like trigeminal neuronal activity and establish the potential of A_3AR as a novel target for the treatment of migraine and related headache disorders.

In male and female Sprague Dawley rats, *in vivo* electrophysiological extracellular recordings of trigeminocervical complex (TCC) neurons were performed, measuring ongoing spontaneous activity, and somatosensory responses to dural electrical stimulation and cutaneous innocuous (brushing) and noxious (pinching) stimulation of receptive fields within the ophthalmic trigeminal division (V1). Under these conditions, we tested the effects of intravenous (IV) administration of the selective A_3AR agonist, Cl-IB-MECA (1mg/kg), naratriptan (3mg/kg; positive control), or saline (vehicle control) on these neuronal outcomes, over 180 minutes.

Cl-IB-MECA and naratriptan significantly decreased TCC neuronal responses to dural electrical stimulation (both A-delta and C fibers responses) and ongoing spontaneous firing, with no effect of vehicle controls (n=12 each). This inhibitory effect was observed starting from 15 minutes following IV administration and lasted for the 180-min recording period, without recovery. Neither CI-IB-MECA nor naratriptan affected responses to cutaneous stimulation, however, Cl-IB-MECA did cause a decrease in blood pressure, likely mediated by off-target effects.

These data provide preclinical support that A_3AR agonism modulates dural-responsive trigeminocervical neurons, inhibiting neuronal activity, and may therefore have a potential role in migraine-related mechanisms. It also provides strong support for A_3AR as a novel target for therapeutic development. Future studies will use new generation, highly selective A_3AR agonists, to confirm the efficacy of A_3AR.

10A

Regulation of the Tie1/Tie2 angiogenic signaling pathway by Angiopoietin-like 4

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Angiopoietin-like 4 (ANGPTL4) is a multifunctional glycoprotein regulating glucose/lipid metabolism, vascular permeability, wound healing, angiogenesis, and tumorigenesis. Unlike angiopoietins, ANGPTL4 doesn't bind to the Tie2 receptor. The angiopoietin/Tie2 pathway plays an important role in maintaining vascular stability, vascular quiescence and promoting angiogenesis. However, the significance of ANGPTL4 in Tie2 signaling is not known.
Here, we examined the combined effect of ANGPTL4 and VEGF on angiogenesis. Our results showed that co-treatment with both factors synergistically enhanced angiogenic potential of the endothelial cells, exceeding the effects of either factor alone. To further elucidate the mechanisms underlying the angiogenic effects of these growth factors, we examined their impact on Tie2 phosphorylation. Our results revealed that both ANGPTL4 and VEGF induced Tie2 phosphorylation. Given that ANGPTL4 does not bind to Tie2, this implies the existence of an indirect mechanism that connects ANGPTL4 to Tie2 signaling pathways. Intriguingly, we found that ANGPTL4 treatment induces Ang1 secretion and also AKT phosphorylation, a key downstream target of Ang1, suggesting that ANGPTL4 may activate similar signaling pathways.

ANGPTL4 rapidly induced Ang1 secretion and Tie2 phosphorylation. Interestingly, inhibition of protein secretion abolished this effect, suggesting ANGPTL4 impact on Tie2 signaling is indirect, via Ang1 secretion. Notably, integrin α5β1 signaling is an established mechanisms that enhance Tie2 sensitivity to Ang1. Our findings reveal that β1 integrin knockdown abrogated ANGPTL4-mediated Tie2 phosphorylation, underscoring the crucial role of integrin signaling in this process. Collectively, we demonstrate that integrin α5β1 signaling and Ang1 secretion may contribute significantly to ANGPTL4-driven Tie2 phosphorylation.

These results provide valuable insights into the role of ANGPTL4 in modulating vascular integrity and stability. It also suggests that Ang1 and Tie2 are novel critical effectors in ANGPTL4 signaling. Future studies are in progress to address how perturbation of ANGPTL4/Tie2 expression affects dysregulated neovascularization and edema in human diseases characterized by pathological angiogenesis.

11A

Functional alterations in PBP4 and GdpP synergistically mediate high-level, broad-spectrum beta-lactam resistance

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The bacteria *Staphylococcus aureus*, a frequent colonizer of humans, can also cause infections such as bacteremia, osteomyelitis or sepsis. *S. aureus* also has the ability to be resistant to antibiotics such as New Generation β-lactams (NGBs), making it one of the leading causes of mortality globally. Mutations resulting in alterations in expression of PBP4 (Penicillin Binding Protein-4), a protein involved in synthesis and maintenance of the cell wall, have been identified as an important, non-classical mediators of resistance. These mutations were recently also detected in natural isolates of *mecA*-deficient, clinically isolated, NGB-resistant strains termed as Methicillin-Resistant Lacking *mec* (MRLM). The mechanisms with which MRLMs mediate NGB resistance is relatively unknown. In this study, we demonstrated the effect of PBP4-associated mutations (regulatory site mutations and/or missense mutations) on the protein expression and function as well as on resistance to a range of antibiotics, including NGBs and vancomycin, using techniques such as cell wall purification and separation, fluorescence microscopy and resistance assays. We also explored the role of PBP4-associated alterations along with altered-functioning of GdpP (a trait often detected in MRLM strains). Abolishment of GdpP activity due to deletion of the gene resulted in increased levels of intracellular CDA and NGB tolerance. We discovered that mutations that alter PBP4 and GdpP functions, which are often present among MRLMs, can synergistically mediate high-level, broad-spectrum resistance to NGBs. Using MIC assay, population analysis and growth assay, our results revealed that this novel mechanism potentially enables MRLMs to produce resistance toward NGBs at levels comparable to those of MRSA strains. Taken together, the findings of this study help us take a step closer towards determining the exact mechanism of resistance seen in MRLM strains and establishes PBP4 as an important mediator of antibiotic resistance.

12A

Immune-driven lipidome remodeling in *Bordetella pertussis* infected nonhuman primate lungs using wide-field MSI

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Introduction

Bordetella pertussis (*Bp*), the causative agent of Whooping Cough, disrupts immune homeostasis through virulent factors like pertussis toxin (PT), impairing immune function and promoting bacterial survival. Phospholipids (PLs) and lysophospholipids (lysoPLs) regulate immune responses, affecting inflammation, signaling, and membrane integrity. During infection, lysoPLs modulate leukocyte recruitment and cytokine production, while PL shifts impact cell activation. Despite its significance, lipid alterations in Bp infection remain unclear. This study uses mass spectrometry imaging (MSI) to examine PL and lysoPL distributions in baboon lung tissues infected with wild-type *Bp* and its ΔPT variant, aiming to elucidate lipid-mediated immune responses.

Methods

Six 6-month-old baboons were infected with *Bp* D420 WT or D420 ΔPT. Animals received 1mL of *Bp* strains in the trachea and 0.5mL in each nostril. After three, seven and fourteen days, one lung per animal (1 per group for each time point) was inflated with 2% gelatin, snap-frozen, and stored at -80°C. Coronal lung sections (13µm) were placed on large-format slides (2x3in). Norharmane matrix (7.5mg/mL) was used, and MSI was performed on a timsTOF Flex in negative ion mode at 30µm spatial resolution.

Results

Lipidomic analysis revealed distinct phospholipid distributions. Phosphatidylinositols (PIs) such as PI 36:3, PI 36:2, and PI 38:4 were localized in inflammatory regions with high bacterial infiltration but were not globally increased in WT tissues. Phosphatidylglycerols (PGs), including PG 34:1, PG 36:2, and PG 36:1, were more evenly distributed and abundant in less inflamed areas. LysoPLs remodeling was higher in WT-infected tissues, with increased LPI/PI and LPG/PG ratios, reflecting enhanced immune activation. The ΔPT group exhibited lower lysoPL levels, indicating a reduced early immune response. These findings suggest PIs are concentrated in inflammatory regions, while PGs associate with less inflamed areas, linking lipid metabolism to immune modulation in *Bp* infection.

13A

Characterization of lipid droplet population dynamics in normal aging and Alzheimer's disease mouse models

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Lipids are a major component of the brain and have been increasingly implicated in the processes of normal cognitive aging and age-related neurodegenerative diseases. While the abnormal accumulation of lipids, originally termed “lipoid granules", was described in Alois Alzheimer’s original report in 1907; to date very limited studies have focused on the role of the lipid droplet (LD) in aging and Alzheimer’s Disease (AD) neuropathology. The aim of this study is to define LD dynamics in aging and evaluate the contribution of apolipoprotein E allele status and amyloid deposits on LD populations in mouse models. Dual polarity MALDI-MSI carried out at 50 µm spatial resolution to achieve subregional analysis of lipids showed differential distribution of several species of PLs, including SM, PC, PA, PE, SHexCer, PS, PI and cholesterol ions in WT and AD mouse models (i.e. 5xFAD, APOE4, APOE3, E4FAD and E3FAD mice) across an aging timeline in the hippocampal formation. Immunohistological (IHC) analysis for lipid droplet (PLIN1/PLIN2), neurons (NeuN), microglia (Iba1), astrocytes (GFAP) and amyloid plaque (Methoxy X04) was carried out post-MSI to associate the regional heterogeneity in lipid composition and LD maps to cellular pathology in the brains. IHC results showed differential expression and distribution of PLIN1 and PLIN2 in the mouse brain. Furthermore, we observed two different subsets of $PLIN1^{+}$ LD populations: one is a bigger cluster that colocalized with amyloid plaque and glial markers (Iba1 and GFAP), particularly in the plaque-dense subiculum and CA1 regions of the hippocampus, and the second population consisted of tiny multifocal punctate in the granular/molecular layer of dentate gyrus that did not colocalize with either amyloid plaques or glial markers. Ongoing studies are underway to characterize the lipid populations of brain LDs separated by sucrose gradient and fine-scale MSI is underway to refine the *in-situ* LD characterization.

14A

TARGETING THE S1P-S1PR1 SIGNALING AXIS IN MIGRAINE-LIKE DURAL-RESPONSIVE TRIGEMINOCERVICAL NEURONS IN RATS

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Emerging evidence links dysregulated sphingolipid metabolism to various clinical pain states through over-production of sphingosine-1-phosphate (S1P) in spinal dorsal horn neurons, engaging S1P receptor 1 (S1PR1). Functional S1PR1 antagonists like ozanimod, approved for MS, show effectiveness in models of persistent neuropathic pain. However, it is unknown if S1P-S1PR1 signaling contributes to trigeminovascular sensitization in migraine-related models.
The objective was to examine the S1P-S1PR1 signaling axis in migraine-related activation and sensitization of trigeminocervical (TCC) neurons.

Using male and female rats, we utilized in vivo electrophysiological extracellular techniques to record dural-responsive TCC neurons in naïve rats, or a model of trigeminovascular sensitization. Here, restraint stress (2h/day for 4 days) was used to mediate latent sensitization that was unmasked by administration of a non-noxious dose of the nitric oxide donor, sodium nitroprusside (SNP, 30µg/kg, IV). We evaluated the acute effects of two functional S1PR1 antagonists, FTY720 (1mg/kg, IV) and ozanimod (1mg/kg, IV), in naïve animals, and the effects of ozanimod given acutely (single dose) or preventively (daily for five days prior to electrophysiology) in the restraint stress/SNP model, measuring neuronal responses over three hours.

Pretreatment with FTY720 (n=7) and ozanimod (n=7) significantly inhibited ongoing spontaneous neuronal firing and intracranial dural-evoked Aδ and C fiber responses in naïve rats. Additionally, acute ozanimod (n=7) reduced the prolonged increase in ongoing TCC neuronal activity and hypersensitive responses to intracranial and extracranial innocuous and noxious V1 cutaneous stimulation induced by restraint stress/SNP. Preventive treatment with ozanimod completely blocked the development of these neuronal outcomes in the restraint stress/SNP model.

These preclinical data show that S1PR1 antagonists (ozanimod and FTY720) modulate the trigeminal ganglion-TCC pathway, attenuating trigeminovascular neuronal activity in a migraine-like model. It also supports the S1P-S1PR1 signaling pathway involvement in migraine activation and sensitization mechanisms, establishing S1PR1 as a potentially novel target for migraine treatment.

15A

Targeting metabolic pathways alleviates bortezomib-induced neuropathic pain without compromising anticancer efficacy in a sex-specific manner

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Abstract

Chemotherapy-induced peripheral neuropathy (CIPN) is a debilitating side effect of cancer treatment that can significantly impact patients' quality of life. This study investigated the effects of targeting metabolic pathways on bortezomib-induced neuropathic pain and tumor growth using a Lewis lung carcinoma (LLC) mouse model, while also exploring potential sex differences in tumor burden and the efficacy of metabolic interventions. Our findings demonstrate that metformin and aerobic glycolysis inhibitors, such as dichloroacetate (DCA) and oxamate, effectively attenuate bortezomib-induced neuropathic pain without compromising the anticancer efficacy of bortezomib in both male and female tumor-bearing mice. Interestingly, the LLC mouse model exhibited a paraneoplastic neuropathy-like phenotype, suggesting its potential as a tool for studying this condition. Significant sex differences were observed in tumor growth, with male mice exhibiting larger tumors compared to females. Furthermore, sex-specific responses to metabolic interventions were noted, with oxamate being more effective in alleviating allodynia in males and metformin and DCA showing greater efficacy in reducing tumor growth in females. These findings highlight the importance of considering sex as a biological variable in preclinical and clinical studies investigating cancer biology, CIPN, and potential therapeutic interventions.

Key words: chemotherapy-induced peripheral neuropathy (CIPN), bortezomib, metabolic interventions, metformin, dichloroacetate, oxamate, Lewis lung carcinoma (LLC), sex differences

16A

Rising Threat of C. auris Incidence and Anti-fungal Resistance at University of Maryland Medical System

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Candida auris is a newly emerging fungal pathogen that causes systemic infections with mortality rates of up to 50% and is categorized as a multi-drug-resistant public health threat by the CDC. In 2021 alone, there was a 95% increase in clinical cases in the United States with 17 states reporting their first *C. auris* case between 2019 and 2021. In this study, we aim to identify current trends of incidences and rates of antifungal resistance of *C. auris* within the University of Maryland Medical System (UMMS).

During a four-year surveillance period (2021-2024), we identified at least 21 patient cases of *C. auris* infections. Phenotypic characterization of recovered isolates demonstrated wide variation in ability to form biofilm and aggregate, two important virulence factors. However, sequencing of the ITS gene identified that 20 of the 21 isolates originated from the same genetic clade indicating intra-clade phenotypic variability. One patient with systemic infection was prospectively sampled over the course of several months of antifungal treatment. *C. auris* isolates later obtained during the infection were not only be more resistant to antifungals than earlier obtained isolates but were also resistant to all antifungal drug classes, suggesting evolution of drug resistance development during therapy. Results from this study indicate a rising threat of *C. auris* warranting accurate monitoring for establishing effective protocols for controlling this serious pathogen.

17A

Developing a Mouse Model of Post-Infectious Irritable Bowel Syndrome following *Clostridium difficile* Infection (Post-CDI IBS)

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Post-infectious irritable bowel syndrome (PI-IBS) is a chronic gastrointestinal disorder affecting up to 30% of patients after an acute *Clostridioides difficile* infection (CDI), significantly impacting their quality of life. However, no animal model exists to study the pathogenesis of the disease or investigate the underlying mechanisms of PI-IBS. To address this, we established a disease model by inducing acute CDI in C57BL/6 mice followed by stress to trigger PI-IBS symptoms, such as chronic gastrointestinal dysfunction, low-grade inflammation, and visceral pain.
Our findings revealed that CDI induced acute colitis characterized by severe diarrhea, weight loss, intestinal inflammation, and tissue damage. After mice recovered from symptoms and cleared bacterial colonization, mild psychological stress (induced by forced swimming) resulted in typical symptoms of IBS, including increased gut motility and permeability in female, but not male, mice. During the disease peak, the expression of tight junction-related genes (*ZO-1*, *Claudin-2*, *Occludin-2*) was significantly reduced in the lower intestines. Histological examinations showed abnormal colonic structures, such as elongated crypts in the distal colon and significant colonic hyperplasia. Elevated fecal lipocalin-2 levels, indicative of gut inflammation, were detected in the late phase of IBS. Importantly, infected mice exposed to stress developed colonic hypersensitivity, a hallmark of IBS.

This study successfully establishes a post-CDI IBS mouse model that mirrors clinical features of human PI-IBS. The model provides valuable insights into its pathophysiology and serves as a tool for investigating disease mechanisms and testing potential therapies.

1B

Resin Coating with Antibacterial and Remineralization Properties to Combat Root-Caries

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Tooth root caries account for 10.1% of all dental caries in the USA. This study developed a multifunctional resin coating with calcium (Ca) and phosphate (P) ion release and antibacterial properties to combat root caries. The effects of nano-sized amorphous calcium phosphate (NACP) and dimethylaminohexadecyl methacrylate (DMAHDM) on mechanical, physical, antibacterial properties against *Streptococcus mutans*, and cytotoxicity on dental pulp stem cells and gingival fibroblasts were evaluated. A coating resin combining urethane dimethacrylate (UDMA), triethylene glycol divinylbenzyl ether (TEGDVBE), DMAHDM, and NACP was synthesized and compared with Seal&Protect and Vanish XT. Experimental groups (UV + 5% DMAHDM + 10%, 15%, and 20% NACP) showed flexural strength (70.9±8.0 to 81.1±6.0)MPa, significantly higher than Seal&Protect (48.2±7.2)MPa (p<0.05) and comparable to Vanish XT (70.2±13.6)MPa, (p>0.05). Elastic modulus (2.2 to 3.3)GPa was lower than Vanish XT (9.4±1.1)GPa (p<0.05). Experimental groups showed 8-log CFU reduction, 96% reduction in metabolic activity and 87% lactic acid production, and increased Ca (1.25±0.03) mmol/L and P (0.8±0.001) mmol/L release over 35 days. Cytotoxicity for experimental groups against dental pulp stem cells and human gingival fibroblast was low and matched those of commercial controls already used in clinic. The resin demonstrated potent antibacterial properties, high ion release, low cytotoxicity, and maintained physical and mechanical integrity, offering potential to prevent root caries formation and progression.

2B

Novel Calcium-Phosphate Cement with Drug Delivery for Bioactive Pulp Capping

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Traditional pulp-capping materials such as mineral trioxide aggregate (MTA) exhibit excellent biocompatibility and sealing performance. However, drawbacks such as prolonged setting time, low bioactivity and high costs persist. Metformin can promote osteodifferentiation and mineralization and may be effective in targeting pulp cells in dentin synthesis.
Objectives: (1) Develop calcium phosphate cement (CPC) with enhanced physio-mechanical characteristics; (2) incorporate metformin into CPC (CPC-Met) and measure its release; and (3) investigate human dental pulp stem cells (hDPSCs) response to CPC-Met.

Methods: CPC was mixed with chitosan at different powder-to-liquid ratios to evaluate physio-mechanical properties compared to MTA. The optimized CPC formulation was loaded with 0, 50, 100, and 150μg of metformin to measure release and assess hDPSCs attachment and proliferation (1, 4, and 7d) via live/dead imaging and SEM. One-way ANOVA was used for statistical analysis. Dissimilar superscript letters in the results indicate statistically-significantly differences ($p<0.05$).

Results: CPC at 3.25:1 ratio significantly reduced the setting time to (41.5±2.1) min versus (123±4.2)min for MTA. Metformin release was proportional to concentration: CPC-150, CPC-100 and CPC-50 released (187.43±15.99) μg/mL, (105.97±16.44) μg/mL and (74.91±5.02)μg/mL, respectively. SEM showed CPC surfaces with pores and well-formed hydroxyapatite nanostructure. Across all groups, the density of live cells exhibited a significant increase over time, indicating active cell proliferation ($p<0.05$). There were no significant differences in either cell density or viability between the groups at any of these time points ($p>0.05$). The percentage of live cells remained consistently high (>93% at 1d, >95% at 4d, and ≈98 % at 7d), with no significant differences observed between all groups, highlighting excellent viability across all experimental conditions.

Conclusion: Novel self-setting CPC-Met shows promise as: (1) fast-setting pulp-capping material; (2) carrier to deliver metformin into the pulp to enhance dentin repair; and (3) excellent mechanical properties and lower cost compared to MTA.

3B

Novel Antimicrobial and Remineralizing Resin-Based Clear Aligner Attachment Orthodontic Materials

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Clear aligner orthodontic treatment provides a more hygienic and esthetic alternative to fixed appliances. Attachments play a crucial role in clear aligner therapy, but prolonged use is associated with plaque accommodation and white spot lesions. The aims of this research were to develop a novel resin-based antibacterial and remineralizing clear aligner attachment material to reduce white spot lesions during treatment while maintaining good mechanical and physical properties. The resin-based clear aligner attachment material was formulated by adding triethylene glycoldivinylbenzyl ether (TEG-DVBE) and urethane dimethacrylate (UDMA), denoted as (UV), along with 3% of the antibacterial monomer dimethylaminododecyl methacrylate (DMADDM), nano-amorphous calcium phosphate (NACP) and glass fillers at different mass fractions (45%,50%,55%). Transbond and Vitremer were selected as commercial controls. Flexural strength, elastic modulus, degree of conversion and microhardness were evaluated. The experimental groups with glass fillers at 45%,50% and 55% exhibited flexural strength from (109.2±8.6) MPa to (96.4±8.2) MPa, exceeding the ISO standard for resin-based materials, but significantly lower than the commercial control (p<0.05). The elastic modulus for the group containing 20% NACP+45% glass was (8.75±0.42) GPa, significantly higher than the other experimental groups (p<0.05). The degree of conversion for all experimental groups with different glass mass fractions (45%,50%,55%) ranged from (53.4±2.3)% to (68.3±0.9)% significantly higher than the commercial control (37.1±4.1)% (p<0.05). Hardness was higher for the 20% NACP+45% glass group at (2.4±0.2) GPa and the 10% NACP+55% glass group at (2.4±0.1) GPa, than the other groups (p<0.05). All experimental groups with 3% DMADDM showed 6-log CFU reduction and 90% reduction of metabolic activity and lactic acid production.

The modified resin based clear aligner attachment material demonstrated excellent antimicrobial activity and remineralizing potential while maintaining good physical and mechanical properties. These qualities may offer enhanced protection against white spots lesion during clear aligner therapy.

4B

Spatial Transcriptomic Characterization of Proliferative Leukoplakia

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Proliferative leukoplakia (PL) is an aggressive high-risk form of oral leukoplakia characterized by multifocality, persistent relentless growth and high rate of malignant transformation to oral squamous cell carcinoma (OSCC) or verrucous carcinoma. The limited predictive value of histopathologic evaluation and lack of traditional environmental risk factors highlight the need to investigate the molecular landscape of PL. Herein, we use spatial transcriptomics to characterize our longitudinal cohort of paired PL cases that transformed to OSCC and those that were stable or progressing, with a focus on areas with host immune response. A total of 8 oral biopsies from 5 different patients were collected. Two pairs of stable/non-transformed PL, 1 pair of transformed PL (PL-OSCC), one PL with oral epithelial dysplasia, and one control sample of OSCC that is not associated with PL. Using FFPE tissue blocks, scRNA sequencing and spatial transcriptomics was performed using Visium HD (10x Genomics). Unsupervised gene clustering resulted in delineated layers of gene clusters in the oral epithelium of our PL samples. We were able to characterize significant genes at different oral epithelial layers (basal/parabasal, spinous, and granular layer). We also noticed sharp interruption of gene clustering at areas with host immune reaction indicating neoantigen pathogenesis with 9 genes potentially involved. The top differentially expressed genes (DEG) between crevicular epithelium (internal control) and PL are S100A8, S100A9, and S100A2. Analysis at malignant transformation zone resulted in 6 DEGs (PI3, SPRR1B, S100A8, S100A9, KRT14, and KRT16). Another observation was FLG gene that was consistently expressed at the top layers of stable PL cases and significantly decreased after malignant transformation indicating a tumor suppressor function and potential target therapy. Our findings offer valuable insights into the pathogenesis of PL and demonstrate that molecular diagnostics using biomarkers is a viable approach for predicting malignant transformation and guiding therapeutic strategies.

5B

Spatial Lipidomic Analysis of Gingiva in mice with periodontitis

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Periodontitis, a chronic inflammatory disease affecting over 47% of U.S. adults, arises from the host immune response to microbial biofilms, leading to destruction of tooth-supporting tissues and an increased risk of systemic diseases. Despite existing treatments, the disease often remains resistant to conventional therapies.

Lipids play a crucial role in cellular signaling, inflammation regulation, and gingival tissue integrity. Disruptions in lipid metabolism contribute to chronic inflammation, yet their precise roles and spatial distribution in periodontium under periodontitis remain underexplored. Lipidomics, a specialized branch of metabolomics, enables comprehensive lipid profiling, offering insights into their role in periodontal inflammation and disease mechanisms.

The aim of my study is to spatially map lipid profiles in gingival tissues from mouse model of periodontitis using mass spectrometry imaging (MSI) to uncover the role of lipids in periodontal inflammation, tissue destruction, and disease progression.

To achieve the aim, a ligature was placed around the second maxillary left molar of three female WT mice, with the right side serving as a control. After seven days, palatal gingival tissues were harvested, snap-frozen in gelatin, and cryosectioned. Spatial lipidomic profiling was performed in negative ion mode by Matrix-Assisted Laser Desorption Ionization MSI (MALDI-MSI). Hematoxylin and Eosin (H&E) staining images were used for histological overlay. The top 100 peaks showing the highest intensities were putatively identified using Alex123, and pair-wise statistical comparisons between ligature and control groups were conducted. Our preliminary analysis showed distinct differences in lipid intensity between the periodontitis (n=3) and control (n=3) groups in epithelium and connective tissue. For example, the ions m/z 713.361, 890.639, 891.642, and 906.635 in the epithelium and m/z 701.511, 890.639, and 906.635 in connective tissue showed statistically significant differences. These findings suggest periodontitis induces changes in gingiva's lipid profiles, warranting further research on the roles of the identified lipids in periodontitis pathogenesis.

6B

Antibacterial and Antifungal Ionic Liquid-Based 3D Printed Resin: Mechanical Properties and Clinical Potential

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Over time, acrylic resin degrades, creating a porous surface that promotes microbial colonization and increases the risk of *Candida*-associated denture stomatitis. This in-vitro study evaluated the antibacterial, antifungal, and mechanical properties of ionic liquids (ILs) incorporated into 3D-printed denture resins.

Antibacterial and antifungal activity of five ILs were tested against *Streptococcus mutans* (UA159) and *Candida albicans* (SC5314) using Minimum Inhibitory Concentration (MIC), Minimum Bactericidal/Fungicidal Concentration (MBC/MFC), and Disc Diffusion assays. The tested ILs included 1-Octyl-3-methylimidazolium tetrafluoroborate (OMIM-BF4), 1-Butyl-3-methylimidazolium tetrafluoroborate (BMIM-BF4), Trioctylmethylphosphonium docusate (TOMP-SCC), 1-Ethyl-3-methylimidazolium acrylate (EMIM-ACR), and 1-Ethyl-3-methylimidazolium vinyl acetate (EMIM-VINYL). EMIM-ACR and EMIM-VINYL were incorporated into 3D-printed resins at 0%, 2.5%, 5%, 7.5%, 10%, and 15%. Mechanical properties were evaluated using degree of conversion (DC%), flexural strength (FS), and elastic modulus. Statistical analysis was performed using two-way ANOVA ($\alpha = 5\%$) with Bonferroni correction.

TOMP-SCC exhibited the strongest antimicrobial activity (MIC: 3 mg/mL for *C. albicans* and >1 mg/mL for *S. mutans*), followed by EMIM-ACR (5.3 mg/mL). A significant interaction between IL type and concentration was observed for FS and modulus ($p<0.05$), highlighting their concentration-dependent mechanical behavior. FS peaked at 10% EMIM-ACR (159.75 MPa, $p<0.05p$) but declined at 15% (114.36 MPa). Modulus increased with EMIM-ACR up to 7.5% (5.98 GPa, $p<0.05p$) but showed no further improvement at 15%. For EMIM-VINYL, FS peaked at 5% (144.72 MPa, $p<0.05p$) before decreasing, while modulus followed a similar trend, peaking at 5% (4.85 GPa) and reaching the lowest value at 15% (2.71 GPa, $p<0.05p$). DC% peaked at 7.5% EMIM-ACR (62.11%) and 5% EMIM-VINYL (61.46%), declining at higher concentrations.
IL-modified 3D-printed resins demonstrate promising antimicrobial potential while maintaining good mechanical properties, emphasizing the importance of optimizing IL concentration for denture applications.

7B

Resin-Based Antibacterial Provisional Crown Coating to Suppress Biofilms and Secondary-Caries

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Abstract: Provisional crowns are often used in dentistry for prolonged periods, but bacterial attachment and dental plaque often lead to gingival inflammation and secondary caries.

Objectives: The aims of this research were to develop a novel resin-based antibacterial provisional crown coating to prevent secondary caries and investigate the physical properties and antibacterial efficacy.

Methods: The resin-based coating was prepared by addition of triethylene glycoldivinylbenzyl ether (TEG-DVBE) and urethane dimethacrylate (UDMA), with the antibacterial monomer dimethylaminododecyl methacrylate (DMADDM). DMADDM was incorporated at mass fractions of 0%, 2.5%, 5%, 7.5% and 10%, creating five experimental groups. TEMPSMART provisional crown material was selected as a commercial control. Surface roughness and contact angle were assessed. *Streptococcus mutans* (*S. mutans*) biofilms on provisional crowns coated with the resin-based coating and cytotoxicity were evaluated.

Results: The surface roughness (0.181±0.041; n=10) R_a,μm of the resin coating with 10% DMADDM was similar across all groups (p>0.05), showing that adding DMADDM did not have a negative impact on surface roughness. The contact angles of 5%, 7.5% and 10% groups were similarly hydrophilic (51.7±7.6; n=15) °, while other groups had significantly more hydrophobic contact angles (70±6.8)° (p<*0.01*), but overall hydrophilicity did not negatively affect the performance of the coating. The incorporation of 5% DMADDM demonstrated a significant antibiofilm effect on *S. mutans* biofilm CFU (n=6) with a 4-log reduction compared to controls (p<0.01) while the 7.5% and 10% DMADDM groups showed 5 and 8-log reduction respectively (p<0.01). Significant reductions of 4-5 folds (p<0.01) were observed in biofilm metabolic activity and lactic acid production (n=6). Incorporating DMADDM did not increase the cytotoxicity toward human gingival fibroblasts as all groups had viability of >82% (p>0.05) (n=3).

Conclusion: The findings suggest that the novel coating material could enhance the long-term performance and clinical outcomes of provisional crowns, contributing to better patient oral health.

8B

METFORMIN ENHANCES VEGF SECRETION IN HYPOXIC DENTAL PULP STEM CELLS

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Objectives:
Regeneration of critical-sized craniofacial bone defects remains a major challenge in oral and maxillofacial surgery. Stem cell-based bone tissue engineering using scaffolds and bioactive factors offers an attractive alternative to autologous bone grafts. Critical to the success of stem cell-based bone regeneration is the survival of transplanted cells within a hypoxic microenvironment, and the formation of a functional microvasculature. This process is mainly controlled by the hypoxia-inducible factor-1a (HIF-1a)/vascular endothelial growth factor (VEGF) pathway, coupling angiogenesis to skeletal regeneration. We have previously reported that metformin, a first-line, low-cost FDA-approved oral antidiabetic drug, induces osteogenic differentiation of dental pulp stem cells (DPSCs). Here, we investigated the impact of metformin on the viability of hypoxic DPSCs and their secreted angiogenic factors.

Methods:
Cell viability assays and immunoblotting were performed in human-derived DPSCs exposed to normoxia (20%O_2) and hypoxia (1%O_2) or HIF-1a chemical stabilizers (i.e., cobalt chloride and dimethyloxalylglycine, DMOG) in combination with metformin (0-50 mM). DPSC-derived conditioned media were used for angiogenesis antibody arrays and ELISA.

Results:
When compared to controls, adding metformin to cells exposed to hypoxia or treated with HIF-1a stabilizers caused no detrimental effect on DPSC viability evaluated at 24-72 hours. Interestingly, we found that metformin combined with hypoxia or HIF-1a stabilizers, significantly upregulated total HIF-1a levels. The impact of HIF-1a upregulation on 55 DPSC-derived angiogenic factors was then explored with angiogenesis antibody arrays. Notably, VEGF was uniquely and markedly increased in conditioned medium from DPSCs concurrently treated with metformin and DMOG versus untreated control or single treatments. Likewise, soluble VEGF levels quantified following ELISA were two-fold higher in DPSCs exposed to hypoxia/metformin than to hypoxia alone ($p<0.0001$).

Conclusions:
Our data indicate that repurposing metformin within DPSC-seeded locally delivered scaffolds may offer a novel approach to pharmacologically enhance HIF-1/VEGF-mediated angiogenic-osteogenic coupling to potentiate vascularized craniofacial bone regeneration.

9B

Barriers to Oral Healthcare for Undocumented Children

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Purpose: This pilot study's purpose is to identify challenges to optimal oral health for undocumented children. The aims are to assess oral health-related quality of life (OHRQoL), determine barriers to obtaining dental care, and characterize oral health attitudes of parents of undocumented children.

Methods: This cross-sectional study administered a written 37-question survey to parents of undocumented children who presented to the University of Maryland Pediatric Dentistry clinic. Questions asked for information demographic information, OHRQoL, barriers to care, and attitudes. Descriptive statistics, Mann Whitney U Test and Kruskal-Wallis Tests were used to evaluate data.

Results: Forty-nine parents participated in this study. The most frequently reported negative OHRQoL indicators were dissatisfaction with esthetics (37%), missed school days (35%) and tooth pain (33%). Negative oral health care attitudes including fears of provider bias (77%), language barriers (46%) and cultural differences (43%). No significant associations were found between housing and food insecurity, duration of residence in the United States, and negative OHRQoL.

Conclusion: Undocumented children experience negative sequelae of poor oral health, difficulties in access to oral health care providers, and concerns of provider bias and lower quality care. Increased knowledge of the oral health experiences, attitudes, needs and social determinants of health of undocumented families is indicated.

10B

Long-Term Impact of Preclinical Distance Learning in Predoctoral Education

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Objectives: Studies investigating the impact of distance learning (DL) on student learning outcomes in dental education are limited. This study aimed to examine the impact of a preclinical DL module on theoretical comprehension and clinical competency in periodontics and fixed prosthodontics through a longitudinal assessment.

Methods: The study included the 2023 cohort (n1 = 126) and the 2024 cohort (n2 = 128). The 2023 cohort experienced a DL module during their second year, followed by onsite learning modules in their third and fourth years. The 2024 cohort underwent onsite learning modules consistently from their second through fourth years. Both cohorts experienced comparable clinical experiences. Assessments in each course were identical or similar for the two classes. Two-way repeated measures ANOVA and linear regression tests were conducted to compare the two cohorts' performances.

Results: The 2023 cohort with preclinical DL demonstrated significantly higher performances in periodontics at all time points. In fixed prosthodontics, while the 2023 cohort performed significantly better than the 2024 cohort on the second-year didactic assessment, there were no differences between the two classes in the third- and fourth-year performances. The 2023 cohort outperformed the 2024 cohort on the 4th-year periodontics competency examination. The 2023 also outperformed the 2024 in assigning stages and grades for periodontitis cases. However, no significant difference was observed in student performance on the 4th-year fixed prosthodontics competency examination between the two classes. Both cohorts showed either very weak or no correlations between second year and fourth-year performances in both courses.

Conclusion: The inconsistent findings and lack of correlations suggest that the effect of different learning modules during preclinical education on students' learning outcomes in periodontics and fixed prosthodontics appears to be limited and insignificant and may not have a long-term impact.

11B

Tooth Loss among Diabetic Individuals at an Academic Institute

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Introduction: While studies have reported that diabetic individuals are at a greater risk for periodontitis and tooth loss, few studies have examined whether periodontal disease is the primary factor affecting tooth extractions among diabetic individuals. This retrospective cohort study aimed to investigate factors affecting tooth loss among diabetic individuals in comparison to nondiabetic ones.

Methods: 490 participants who had tooth extractions performed by dental students in the undergraduate oral surgery clinic were included. Third-molar and orthodontic extractions were excluded. Patient- and tooth-related data were collected. Severe bone loss with insignificant caries and/or endodontic conditions was categorized as periodontal reason for extraction. Descriptive statistics and a general linear analysis were conducted to investigate the association between the remaining teeth number and selected predictors—age, periodontitis, smoking, and diabetes.

Results: The study population included 100 diabetic (20.4%) and 390 nondiabetic individuals. Our diabetic group exhibited a mean HbA1c value of 7.5%. The diabetic group was significantly older ($p < 0.01$), exhibited more periodontitis ($p < 0.01$), and retained significantly fewer teeth (16 versus 19, $p < 0.01$) compared to the nondiabetic group. 443 participants (90.4%) lost their teeth due to nonperiodontal reasons. There was no difference in the reasons for tooth extractions between the two groups ($p = 0.19$). When age, periodontitis, smoking, and diabetes were considered together, age ($p < 0.001$) and smoking ($p < 0.001$) significantly affected the remaining teeth number, but diabetic status ($p = 0.15$) and periodontal diagnosis ($p = 0.82$) did not affect the number of remaining teeth.

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

12B

Dental Providers' Perceptions Toward Mental Health Screening at Routine Practices

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Objectives: Although individuals struggling with mental health (MH) issues often do not have regular visits with primary care or MH providers, many of them receive consistent dental care. This study aimed to assess the feasibility and barriers to conducting MH screening in dental settings using mixed methods.

Methods: A survey questionnaire was distributed to dental practitioners in the Maryland Practice-Based Research Network. It included questions pertaining to participants' demographic information and questions covering topics such as psychosocial stress, dental practices for MH screening, dental providers' comfort levels, and barriers for MH screening. Descriptive statistics were conducted.

Results: 48 dentists and 20 dental hygienists completed the questionnaire. 49% of participants were female. Most participants were age ≥ 45 years old with > 20 years of experience in private general practice. 84% of participants observed patients experiencing psychosocial stress and 78% noticed an increase in stress during the COVID-19 pandemic. While 62% of participants perceived MH screening at dental settings as feasible and 59% felt comfortable providing MH screening, only 35% were willing to pilot test MH screening. The primary barrier was the scope and business of the dental practice, followed by concerns about patient comfort and a lack of training and knowledge in MH screening.

Conclusions: Future efforts to integrate MH screening into dental practices should address the identified barriers and leverage the dental providers' positive perceptions of feasibility and comfort in MH screening.

13B

Discrepancy between Knowledge and Confidence Levels toward Obstructive Sleep Apnea Among Dental Faculty and Students

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This study aimed to assess the knowledge and attitude toward obstructive sleep apnea (OSA) among pre-doctoral dental faculty and senior dental students to investigate the need for developing a dental sleep medicine curriculum.

The OSA Knowledge and Attitude survey was administered to potential participants at the University of Maryland School of Dentistry (UMSOD). Descriptive statistics were performed to compare performances in the OSA knowledge test between the dental student and faculty groups. Correlation tests were conducted to examine relationships between the knowledge scores and attitudes toward OSA.

51 seniors and 30 faculty members participated in this study. The mean total score in the knowledge test was 12 out of 18 (67%); there was no significant difference in the mean score between the two groups. Both groups perceived that OSA and identifying patients with OSA were very important or important. While most participants reported that they were not confident in managing OSA or patients on continuous positive airway pressure, negative correlations between the knowledge scores and confidence in managing OSA (r_s = -0.3, p = 0.012) and the importance of OSA (r_s = -0.3, p = 0.004) were observed.

Pre-doctoral faculty and seniors at UMSOD exhibited low levels of knowledge and confidence in managing OSA patients, yet both groups recognized the importance of OSA and identifying affected patients. These findings highlight the need for integrating an OSA course into the curriculum and for enhancing faculty training.

14B

Nociceptor neurons facilitate orthodontic tooth movement via Piezo2 in mice

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Multiple sensory afferents, including mechanosensitive and nociceptive nerves, are projected to the periodontium. Peptidergic afferents expressing TRPV1, a receptor for capsaicin, mediate pain caused by orthodontic forces. However, their role in orthodontic, force-induced alveolar bone remodeling is poorly understood, nor is the contribution of mechanosensitive ion channels such as Piezo2 in nociceptive nerves. To investigate this role, we studied orthodontic tooth movement and alveolar bone remodeling using neural manipulations and genetic mouse models. Chemical ablation of TRPV1-expressing afferents localized to the trigeminal ganglia decreased orthodontic force-induced tooth movement and the number of osteoclasts in alveolar bone on the compression side. The extent of force-induced increase of the ratio of receptor activator of nuclear factor kappa-B ligand/osteoprotegerin in the periodontium was modestly decreased in the chemical ablation group. Furthermore, chemogenetic silencing of TRPV1-lineage afferents reduced orthodontic tooth movement and the number of osteoclasts. Piezo2 was expressed in the majority of the periodontal afferents, and chemogenetic inhibition of Piezo2-expressing neurons decreased orthodontic tooth movement and the number of osteoclasts. In addition, the conditional knockout of Piezo2 in TRPV1-lineage afferents decreased orthodontic tooth movement and the number of osteoclasts. Overall, these results suggest that nociceptor neurons play critical roles in orthodontic, force-induced alveolar bone remodeling and that the mechanical activation of neuronal Piezo2 in nociceptive nerves facilitate orthodontic tooth movement and associated alveolar bone remodeling.

15B

Effectiveness of Posterior Ceramic Crowns at an Academic Institute: A Split-Mouth Retrospective Cohort Study

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This split-mouth retrospective cohort study aimed to investigate the effectiveness of posterior ceramic crowns fabricated using digital technology compared to ones fabricated using the impression with elastomer and casting method at an academic institute.

Among 45 potential participants having both ceramic and cast crowns from 2015 to 2023, 31 participants were included after excluding anteriors (7), <1-year follow-ups (5), and bridge abutments (2). Patient- and tooth-related data were collected. Wilcoxon signed-rank, chi-square, and the log-rank tests were conducted to compare the ceramic and cast crown groups.

For the study 15 men and 16 women were included with 62 teeth. The mean observation years did not differ between the ceramic (3.3) and cast (4) groups ($p = 0.123$). There were no differences in the distributions of root canal treatments, clinical crown lengthening procedures, and post-insertions ($p > 0.05$), nor in the probability of tooth survival ($p = 0.14$) between the two groups. The insertion of the ceramic crowns was significantly expedited from making the final impression compared to the cast crowns (21 versus 41 days; $p = 0.0006$). There were no significant differences in the complication rates and tooth loss between the two groups ($p > 0.05$). Most teeth extractions were because of endodontic reasons.

The ceramic crowns demonstrated notable effectiveness over the cast crowns, completing the procedure in significantly fewer days, while resulting in a comparable prognosis with similar complication rates compared to the cast crowns.

16B

Pediatric Dentistry Program Directors as stakeholders in the process of Board-certification

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***Purpose/objectives**:* Board certification is an important credential for pediatric dentists to achieve for purposes of career advancement, hospital privileges, and reputation. The purpose of this pilot study was to assess the engagement of residency Program Directors (PDs) with the Board certification process in terms of criteria for clinical readiness, curriculum development, and value. An additional objective was to identify challenges and ways in which PDs may need support as they prepare residents for Board certification.

***Methods**:* Eighty-seven PDs were invited to participate in this study, out of which 32 agreed to participate (36% response rate). The study included a digital non-validated survey and verbal interviews. Digital and verbal survey results were analyzed using descriptive statistics.

***Results**:* PDs had recurrent themes in their descriptions of clinical readiness, including information gathering, application of knowledge, and professional awareness. Half of the participants used the American Board of Pediatric Dentistry (ABPD) exam domains as a guideline in their curriculum development. Of the verbal interview participants (N = 32), 30(94%) felt that Board certification was very important to their residents and program. Many barriers to successful completion of the Board exam were discussed, and a diverse array of suggestions were made for mechanisms to better support PDs as they prepare residents for
Board certification.

***Conclusions**:* Board certification is valued by PDs and influences their criteria for clinical readiness. Increased engagement with the ABPD will facilitate better support for PDs and will enhance the consistency of training standards in residency programs.

17B

Association of Primary Teeth Contact Type and Caries Status in Children

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Dental caries in children is the most common infectious disease in the US and contributing by complex factors including both personal (Socioeconomical Status, Environmental, and Behavioral) and tooth-specific (Plaque, Diet, Fluoride) factors. However, no single factor was found reliably predicting future caries status and be used as a simple caries risk assessment tool for clinicians. Previous retrospective studies suggested the broad contacts between primary molars strongly associated with caries experience in children. The purpose of this study was to evaluate the relationship between primary molar contact type and the change of caries status over 6 months in pediatric population as well as other risk assessment variables (fluoride, diet, plaque scores). Healthy children aged 3-10 years of age were enrolled. Clinical examination was completed to evaluate caries status, plaque score and contact types between primary molars at the baseline and 6-month follow-up. Fluoride level and diet information were also collected. Multivariate linear regression was used to analyze all the variables. Statistical significance was set at $p < 0.05$. Of the 108 patients that were included at the baseline, only 21 patients presented for 6-month follow-up. The mean age at baseline was 6.1 years. The contact type was not significantly associated with the change in dmft at the follow-up as well as the fluoride and diet. Change of plaque index on mandibular teeth was not significantly correlated, however the change of plaque index for maxillary teeth was found to be significantly associated with dt ($P = 0.0177$). While there are many contributing factors to the development of caries, one localized factor on the tooth level (contact type) doesn't not seem to associate with overall change of the caries status in 6 months for children. Large-scale studies with more subjects will be needed to confirm the findings.

18B

Genetic Downregulation in Subgingival Plaque of Adolescents and Young Adults with Grade C Periodontitis

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Grade C molar-incisor pattern periodontitis (C-MIP) is a severe form of periodontitis in systemically healthy adolescents and young adults. It is characterized by rapid tissue destruction without the presence of heavy plaque and has a familial component. Certain oral pathogens have been associated with C-MIP, but the detail mechanism of microbial pathways is still unclear. Metatranscriptomics were used to analyze subgingival plaque from patients with C-MIP to analyze global gene expression and determine gene function within the biofilm. Upregulated genes have endured the focus of the literature while downregulated genes remained under-explored. The objective of this investigation was to evaluate the genes downregulation and the contributing bacteria of the subgingival microbiomes in healthy versus diseased sites in young adults with C-MIP. Paired subgingival plaque samples were collected from healthy and diseased sites from 12 patients. Total RNA was extracted and RNASeq was performed using Illumina HiSeq2000. Down-regulated genes and associated bacterial species were identified using MTSv pipeline, DESeq2, and apeglm. Significant gene transcripts (KOs) were further mapped using KEGG database to determine the global metabolic pathways. In periodontal pockets, 309 genes were found significantly downregulated. Metabolism was the most frequently downregulated global function, followed by environmental information processing however many the genes found not associated with any metabolic pathways. Among the downregulated metabolic genes, the carbohydrate metabolism and xenobiotics degradation and metabolism were the most frequently related functions. *Paraburkholderia fungorum* and *Sphingomonas sp. FARSPH* were the top two bacterial species associated with the majority of the downregulated genes. The results from this study demonstrated the differential microbial gene expression in periodontal pockets versus healthy sites. The detail roles of the associated bacterial species during the disease development will be needed to investigate by future research using a larger sample size.