1A

Potential Causal Role of Periodontitis in Inflammatory Bowel Disease

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Objective: Emerging evidence suggests an association between periodontitis and inflammatory bowel disease (IBD); yet the underlying mechanisms remain poorly understood. We sought to elucidate the causative role of periodontitis in IBD using translational clinical and animal studies.

Methods: We conducted a case-control study assessing the periodontal status of 180 IBD patients and 180 healthy controls. Each subject underwent a comprehensive oral examination and provided saliva and fecal samples. To mimic human conditions, we induced periodontitis in mice using ligature-induced periodontitis (LIP) and oral gavage models, utilizing mice with inherent gut barrier defects or gut barrier weakened by piroxicam medication.

Results: IBD patients exhibited a higher prevalence of periodontitis compared to healthy controls, notably in the 35-50 and 51-65 age groups. Severe periodontitis and higher bleeding scores were positive risk indicators for IBD. 16S rRNA gene sequencing identified a higher presence of oral taxa in the feces of IBD patients with periodontitis compared to healthy controls. Corroborating these human findings, LIP in mice facilitated the colonization of native oral bacteria in the intestine, leading to colitis. Similarly, oral gavage of human periodontal pathogens induced colitis in mice with gut barrier deficiencies, compared to untreated mice or those inoculated with oral commensals. Oral pathogens promoted infiltration of IgG+ B cells into the colonic lamina propria and increased IgG activation, which triggered elevated IL1b and IL-17 production leading to induction of colitis. Remarkably, the resolution of LIP in mice diminished the intestinal load of oral bacteria and reversed colitis in mice.
Conclusion: The oral cavity may serve as a reservoir for bacteria that can induce colitis via B-cell mediated responses, especially in the presence of compromised intestinal barriers. Dental care can reduce the risk of IBD by limiting the influx of colitogenic oral pathogens and should be considered as an integral part of IBD evaluation and care.

2A

Genetic Access to Trigeminal Primary Afferents Transducing Mechanical Allodynia in Mice

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Trigeminal neuropathic pain interferes with critical functions such as eating and speaking *and* is often resistant to conventional therapy. One of the most debilitating symptoms is allodynia to innocuous mechanical stimuli, such as gentle brushing. However, the identity of trigeminal primary afferents transducing mechanical allodynia is not fully understood, which poses a hurdle to develop novel peripherally targeted analgesia.

Our study demonstrates that in mice with chronic constriction injury of the infraorbital nerve (ION-CCI), facial brushing induced a transient upregulation of *Fos* transcript in a subset of trigeminal ganglia (TG) neurons. Most *Fos*+ neurons were colocalized with markers of low threshold mechanoreceptors, such as *Ntrk2*, *Ntrk3*, and *Mafa*. Interestingly, a subset of *Fos*+ neurons were also colocalized with markers of nociceptors, such as *Calca* and *Mrgprd*. We determined if *Fos* upregulation can be used for genetic access to brushing-activated neurons by adopting Targeted Recombination in Active Populations (TRAP) methods using Fos^{CreER} mice. Chemogenetic inhibition of the facial brushing-mediated TRAPed TG neurons inhibited both punctuate and dynamic mechanical allodynia, whereas chemogenetic activation of the brushing-TRAPed neurons increased the duration of spontaneous face-wiping behavior in mice with ION-CCI. Brushing-induced conditional knockout of Piezo2 from trigeminal afferents reduced punctate and dynamic mechanical allodynia. In vivo GCaMP imaging in intact TG showed that conditional brushing-TRAPed knockout of Piezo2 reduced trigeminal neuronal hypersensitivity to Von Frey stimulation or brushing on the face in mice.

In conclusion, our findings suggest that face brushing-induced *Fos* upregulation can be a useful marker allowing genetic access to allodynia-mediating TG neurons after trigeminal neuropathy.

3A

ENPP1 Roles in Cementogenesis

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Proper regulation of pyrophosphate (PPi), a direct inhibitor of hydroxyapatite crystal growth, is critical in mineralization. PPi can be generated from ATP, which is hydrolyzed by ectonucleotide pyrophosphatase phosphodiesterases (ENPP), including ENPP1. ENPP1 is expressed in bone and cementum, and when mutated or lost, results in skeletal and dental abnormalities. Notably, ENPP1 has been linked with Generalized Arterial Calcification of Infancy (GACI), a severe mineralization disorder characterized by vascular and joint calcifications. Notably, hypercementosis has been observed in individuals with GACI and murine models lacking ENPP1 function, yet the roles of ENPP1 domains/functions in cementogenesis remain unclear. In previous studies, we demonstrated the importance of PPi in cementogenesis; mice lacking ENPP1 function ($ENPP1^{asj}$) exhibit low plasma PPi levels and hypercementosis. To further define ENPP1 roles, we employed two additional ENPP1 mutant murine models with low PPi levels: ENPP1 mutants with targeted loss of ATP hydrolysis ($ENPP1^{T238A}$) or loss of ENPP1-mediated cGAMP hydrolysis ($ENPP1^{H362A}$). We found that $ENPP1^{T238A}$ mice exhibited hypercementosis and ectopic joint calcifications, albeit to a lesser extent compared to $ENPP1^{asj}$ mice. $ENPP1^{H362A}$ mice did not exhibit ectopic calcifications, and in approximately 50% of mice analyzed, exhibited a mild hypercementosis. QPCR was conducted on RNA extracted from periodontal ligament samples of $ENPP1^{asj}$, $ENPP1^{T238A}$, and $ENPP1^{H362A}$ mice. Compared to WT, $ENPP1^{T238A}$ and $ENPP1^{asj}$ mice exhibited higher *progressive ankylosis protein,* another PPi regulator, (3 and 4 fold, respectively) expression, which was unchanged in $ENPP1^{H362A}$ mice. *Spp1,* which encodes osteopontin (an extracellular matrix protein), was upregulated by approximately 30-fold in $ENPP1^{asj}$ mice and approximately 2-fold in $ENPP1^{T238A}$ and $ENPP1^{H362A}$ mice compared to WT. These results demonstrate the importance of ENPP1-mediated ATP hydrolysis and the existence of other ENPP1 contributions in cementogenesis. Future studies will be aimed toward understanding ENPP1-mediated ATP hydrolysis at the cellular level and ATP dynamics in cementoblast mineralization.

1B

Phospholipid Alterations in Different Infection models: insights from mass spectrometry imaging

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Phospholipids are essential components of cellular membranes and play a crucial role in immune regulation during infections. Changes in phospholipid composition and distribution can influence inflammation, signaling pathways, and tissue homeostasis. During infection, host-pathogen interactions trigger lipid remodeling, which can either exacerbate inflammation or contribute to immune resolution. The ability of bacterial and viral pathogens to manipulate lipid metabolism affects disease severity, tissue integrity, and immune cell recruitment. Recent advances in mass spectrometry imaging (MSI) allow for high-resolution spatial mapping of lipid alterations in infected tissues, providing critical insights into disease progression. This study explores phospholipid alterations in different lung infection models, using MSI to characterize lipidomic remodeling during bacterial (*Bordetella pertussis* (Bp) and *Pseudomonas aeruginosa* (*Pa*)) and viral (SARS-CoV-2) infections. By understanding the dynamics of phospholipid changes, we can identify potential biomarkers for disease progression and therapeutic intervention strategies. Lung tissues were collected from *Bp*-infected baboons, *Pa*- and SARS-CoV-2-infected mice at different time points. The samples were gelatin inflated and snap-frozen, sectioned (10-13µm), Norharmane matrix at 7.5mg/mL was applied, and imaging was performed in negative ion mode at 20-30µm spatial resolution on a timsTOF Flex. Distinct phospholipid remodeling patterns were observed across infection models. *Bp*-infected lungs showed localized accumulation of specific phospholipid species in inflammatory regions, while other phospholipids were more evenly distributed in less inflamed areas. In the mice infection model, phospholipid intensities were reduced compared to controls, with distinct spatial clustering forming molecular fingerprints unrelated to standard histological features. Temporal changes in phospholipid distribution were evident, suggesting a dynamic remodeling process influenced by infection type and progression. Phospholipid alterations vary across animal models, bacterial or viral lung infections, reflecting differences in immune activation and tissue responses. These findings emphasize the role of lipid metabolism in infection dynamics and highlight potential lipid-based therapeutic targets.

2B

An Introduction to Artificial Intelligence (AI) in Dental Education and Research

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Artificial Intelligence (AI) is rapidly transforming various domains, including healthcare and dental education. The field of dentistry stands to benefit significantly from the integration of AI technologies to enhance learning, research, and clinical care. This presentation provides an introduction to AI in dental education and research, highlighting key developments and potential applications. Our research from the Division of Artificial Intelligence Research at the University of Maryland School of Dentistry explores the use of language models or 6 AI chatbots (Bing, GPT-3.5, GPT-4, Google Bard, Claude, Sage) responses to controversial and difficult questions in oral pathology, oral medicine, and oral radiology. GPT-4 demonstrated the highest performance across disciplines, achieving a mean score of 4.066. However, accuracy of citations generated by the chatbots remains a challenge, with 23.50% of citations being fake or synthetic. In the context of oral oncology, GPT-4 exhibited the most impressive responses to frequently asked questions (FAQs) by patients. While the AI chatbots showed promise in guiding patients on common oral cancer topics, they underperformed in terms of empathy and citation accuracy. Further research is needed to refine these AI models for reliable patient education and support. We also discuss our recent paper in Modern Pathology, that focuses on leveraging generative AI techniques, such as Generative Adversarial Networks (GANs), to create pseudorealistic histopathology images for rare disease datasets. We importantly discuss the ethical considerations of using synthetic data in academia and research. We also highlight the potential use of large language models (LLMs) to generate AI-powered educational podcasts, aiming to make dental education more engaging and accessible for the newer generation of dental students and residents that are more receptive to podcasts as compared with traditional learning methods. In conclusion, this presentation highlights the potential of AI in revolutionizing dental education and research. However, it is crucial to address the limitations and ethical considerations surrounding AI implementation to ensure its responsible and effective integration into the field.

3B

Induced glycation decreases damping behavior in dentin extracellular matrix

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Advanced Glycation End Products (AGEs) accumulate in collagen inducing cross-links and protein adducts, which ultimately increase tissue stiffness, modify molecular recognition, and impair function and self-repair. AGEs formation is due to non-enzymatic reactions between reducing sugars and amino groups and is associated with chronological aging, systemic pathological diseases, such as type 2 diabetes mellitus. The aim of this study is to assess the effect of induced glycation on the viscoelastic properties of the dentin extracellular matrix (ECM) using dynamic mechanical analysis (DMA).

Mid-coronal dentin was sectioned for micro-DMA and nano-DMA. Demineralized dentin (0.5 M EDTA, pH 8.0) was immersed in 1M d-Ribose for in vitro induced-glycation. Viscoelastic properties were assessed using strain and frequency sweep tests under controlled conditions at different hierarchical levels. Damping capacity (tan δ), storage (E'), loss (E") and complex (E*) moduli were calculated. Pentosidine quantification, a well-known AGEs, was calculated with fluorescence spectrophotometer. Glycated and control specimens were pulverized, hydrolyzed and analyzed. Data were statistically analyzed using one-way ANOVA and post-hoc tests ($\alpha=0.05$)

Induced glycation increased E' while decreasing damping of dentin ECM, revealing a more elastic-like behavior compared to the control, regardless of whether micro- or nano-DMA was used ($p<0.001$). High pentosidine concentration was found in glycated dentin when compared with control group ($p<0.001$).

Multi-scale dynamic mechanical analyses revealed significant shifts in the chemo-mechanical characteristics of the dentin ECM due to the presence of AGEs. Ongoing research explores how AGEs accumulation in individuals with diabetes affects the dentin-pulp complex's biomechanics and function, aiming to establish correlations between higher AGEs concentration, reduced dentin toughening mechanisms and the downregulation of reparative and protective functions in dental pulp cells. Findings will support the exploration of mechanotherapy strategies aimed to restore dentin viscoelasticity and enhance pulp cell responses.