

Office of Research | Continuing Dental Education | American Student Dental Association featuring poster presentations, two CE lectures and the ASDA vendor fair

SPRING SYNERGY

RESEARCH DAY MARCH 29

Health Professions, Nursing & Pharmacy (HPNP) Auditorium

Schedule of Events

7:30 a.m.	POSTER SET-UP	HPNP Nursing Hallway
8:00 a.m.	REGISTRATION AND BREAKFAST	HPNP Reception Hall
8:30 a.m.	KAPLAN SCHOLAR ▶ Aligners: Past, Present and Future	HPNP Auditorium 1404
10:30 a.m.	NETWORKING BREAK	HPNP Reception Hall
10:45 a.m.	RESEARCH KEYNOTE Clinical Research - Greg's Greatest Hat 	HPNP Auditorium 1404
11:30 a.m.	VENDOR FAIR	BMS Building Lobby
11:30 a.m.	POSTER JUDGING / JUDGES ONLY	HPNP Nursing Hallway
12:30 p.m.	LUNCH	HPNP Courtyard
1:30 p.m.	POSTER PRESENTATIONS	HPNP Nursing Hallway
3:30 p.m.	RESEARCH DAY ADJOURNED	Thanks for coming!





GATOR DENTIST DR. GREG HUANG

UFCD Class of 1987 Robert L. Kaplan Scholar and Research Keynote

Chair of Orthodontics & Associate Dean for Faculty University of Washington School of Dentistry

The Robert L. Kaplan Scholar presentation is annually supported by the Robert L. Kaplan Visiting Professorship Fund.



SPRING SYNERGY RESEARCH DAY Keynole Presentation



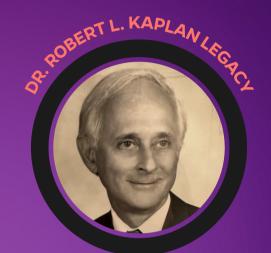
DR. GREG J. HUANG

Aligners: Past, Present and Future Clinical Research - Greg's Greatest Hits

Chair of Orthodontics Associate Dean for Faculty University of Washington School of Dentistry

Dr. Huang graduated from the University of Florida College of Dentistry in the DMD Class of 1987. He went on to specialize in orthodontics, earning a certificate and master's degree in orthodontics from the University of Washington, while also earning his MPH in epidemiology from UW. He's been an academician since 1999 and is the Kokich-Shapiro Endowed Professor, chair of orthodontics and associate dean for faculty at UW. A proponent of evidence-based orthodontics, Dr. Huang is a Diplomate of the American Board of Orthodontics and is heavily involved in clinical research. He's co-editing two well-known orthodontic textbooks. Orthodontics: Current Principles and Techniques, 7th Edition, and Evidence-Based Orthodontics, 2nd Edition.





Dr. Robert L. Kaplan Legacy

Spring Synergy and the Robert L. Kaplan Scholar presentation are annually supported by the Robert L. Kaplan Visiting Professorship Fund. Robert L. Kaplan, D.D.S., past president of the American Academy of Restorative Dentistry, was the chairman of the Dental Advisory Committee in 1969 that was instrumental in making the case to the Florida Legislature for a dental school in the state of Florida, thus playing a critical role in establishing the UF College of Dentistry. The South Florida Dental Association sponsored a campaign to establish the visiting professorship honoring Dr. Kaplan after he passed away in 1989. Dr. Kaplan was the keynote speaker at our college's first commencement ceremony in 1976, and a copy of his speech can be read on www.dental.ufl.edu.

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SPRING SYNERGY RESEARCH DAY Doster Dresentations



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SPRING SYNERGY RESEARCH DAY Doster Dresentations



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2024 SPRING SYNERGY JUDGES

- Dr. Nader Abdulhameed
- 🕨 Dr. Maria Aguilar
- 🕨 Dr. Eleonora Bakiri
- Dr. Tamires Cordeiro
- Dr. Louise Dornelas-Figueira
- Dr. Nicole Fiallos
- 🕨 Dr. Bruna Garcia
- Dr. Hind Hussein
- 🕨 Dr. Nadim Islam
- 🕨 Dr. Jessica Kajfasz
- 🕨 Dr. Nadeem Khan
- 🕨 Dr. Tumader Khouja
- Dr. Dan McNeil
- 🕨 Dr. Surabhi Mishra
- Dr. Dayane Oliveira
- Dr. Patricia Pereira
- **Dr.** Edna Perez
- 🕨 Dr. Roberta Pileggi
- 🕨 Dr. Fernanda Rocha
- Dr. Mateus Rocha
- Dr. Jean-Francois Roulet







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PRE-DENTAL/UNDERGRAD

ABSTRACT PRESENTATIONS







Poster Presentation

Division

Pre-Dental/Undergraduate Student

Abstract Title

Association of Cbp+ Streptococcus mutans with Root Caries in Older Adults

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Authors

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Area of Interest

Clinical Science Research

Abstract

Objective: Root caries is an oral condition that disproportionately affects older adults due to factors such as the increased incidence of root surfaces exposure caused by gum recession with subsequent plaque accumulation. Streptococcus mutans is a key pathogen in the caries process, directly associated with its initiation and development. Nearly 20% of S. mutans strains express collagen-binding proteins (Cbps), specifically Cnm and Cbm, that mediate binding to collagen-rich surfaces. The purpose of this study is to investigate the association of the CBPs in S. mutans with root caries.

Methods: In this cross-sectional clinical study (IRB202100916), we enrolled eligible subjects over 65 years old who were receiving treatment at the University of Florida College of Dentistry. Subjects were grouped as healthy (H) or root caries (RC) based on their oral health status. H subjects presented exposed root surfaces but no presence of active caries lesions and RC subjects presented at least one non-carious, exposed root surface and one active, cavitated root caries lesion. Saliva and supragingival plaque from sound enamel, non-carious root surface, and active root carious lesions were collected from each subject and screened for presence of S. mutans by PCR using S. mutans-specific primers. For colonies identified as S. mutans, the presence of genes coding for Cbps were determined by PCR using cnm and cbm-specific primers.

Results: When comparing the prevalence of S. mutans in oral samples of 87 subjects (43 H and 44 RC), S. mutans was detected in 83.7% of H and 90.91% of RC subjects. RC subjects presented higher prevalence of Cbp+ S. mutans (34%) compared to H subjects (16.2%) (p=0.05). Based on prediction models, individuals infected with Cbp+ S. mutans have 2.6 times the odds of developing root caries compared to those infected with Cbp- S. mutans (p=0.05).

Conclusion: Although the prevalence of S. mutans was similar among healthy and root caries subjects, our findings demonstrate that individuals infected with Cbp+ are at greater risk of developing root caries

than those infected with Cbp- S. mutans.

Poster Presentation

Division

Pre-Dental/Undergraduate Student

Abstract Title

Glucose-PTS Regulates Glycerol Metabolism and Hydrogen Peroxide-Mediated Competition by Streptococcus sanguinis

Presenter' Name:

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Authors

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Area of Interest

Basic Science Research

Abstract

Objectives. As a fermentation byproduct from certain yeast and bacteria, glycerol's contribution to microbial homeostasis in the oral cavity remains to be determined. Previous research on streptococci, which make up the bulk of the supragingival microbiome, has suggested that most do not catabolize glycerol. However, several recent studies have indicated that glycerol is involved in streptococcal interactions with other members of the oral microbiome, and we recently found that the glycerol phosphorylation (glp) pathway was the most highly increased in expression of the entire transcriptome of a glucose: phosphotransferase system (PTS) mutant in comparison to its wild-type parent. Here we examine the contribution of glycerol metabolism to fitness and competition in S. sanguinis and its regulation by carbon catabolite repression (CCR).

Methods. Genetic mutants of the glucose-PTS (manL), glycerol metabolic pathway, and putative transcriptional regulators were constructed and characterized for their ability to catabolize glycerol, for fitness, and for hydrogen peroxide (H2O2) production, backed by transcription analysis, biochemical assays, plate-based antagonism, and mixed-species liquid competition assays.

Results. H2O2 assays identified the glp pathway as a novel source of H2O2 production by S. sanguinis that is independent of pyruvate oxidase (SpxB) activity. Genetic analysis suggested that the glp pathway is regulated by the glucose-PTS (ManLMNO)-mediated mechanism instead of the catabolite control protein CcpA. Conversely, deletion of manL or ccpA each resulted in increased expression of spxB and a second, H2O2-non-producing glycerol metabolic pathway (dehydrogenation, dha), indicative of a mode of regulation consistent with conventional CCR. During a plate-based antagonism and a liquid-based

competition assay, glycerol greatly benefited the competitive fitness of S. sanguinis against S. mutans.

Conclusions. Our study suggests that glycerol metabolism plays a more significant role in microbial ecology than previously understood. Genetic mechanisms controlling expression of the glp pathway in S. sanguinis significantly deviate from that in paradigm Gram-positive species Bacillus subtilis. Commensal streptococci, though not able to use glycerol as a sole carbohydrate for growth, benefit from presence of glycerol through production of both ATP and H2O2.

Poster Presentation

Division

Pre-Dental/Undergraduate Student

Abstract Title

Reposition of FDA-approved antidepressant, 2-PCPA, to treat periodontitis.

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Authors

Anh Hao Dang, Hong Huang, Jia Chang

Area of Interest

Clinical Science Research

Abstract

Background:

Trans-2-Phenylcyclopropylamin (2-PCPA), a long-standing antidepressant monoamine oxidase inhibitor, has been repurposed as an epigenetic inhibitor targeting Lysine-Specific Histone Demethylase 1A (LSD1) for leukemia treatment. Our previous research proved 2-PCPA as a bone growth inducer, preventing inflammatory bone loss induced by cytokine (TNF- α) and cigarette smoking extract in osteoblast cell cultures in vitro and mouse calvaria organ cultures ex vivo. Systemic administration of 2-PCPA improved periodontal jawbone quality in mice.

Objectives:

This study explores the local application of 2-PCPA in ligature-induced periodontitis mice.

Material and Method:

1. UF IACUC (#IACUC202200000355) approved our study.

2. Ligature-Induced Periodontitis Model: We induced periodontitis lesions in 10-11 weeks C57BL/6 mice by placing 5-0 silk sutures between the 1st and 2nd maxillary molars for 12 days.

3. Local 2-PCPA Delivery: 30 µl methylcellulose gel (Sigma-Aldrich, St. Louis, MO) containing 10 mg/kg 2-PCPA or PBS control was applied to periodontal/peri-implant pockets under the isoflurane inhalation anesthesia with microinjection syringes (Hamilton, Reno, NV). The study included two approaches. 1). 2-PCPA was applied during ligature placement every other day (total 6 times) to prevent disease and bone damage. 2). 2-PCPA was applied every other day (total 6 times) after ligature removal to treat the established periodontitis lesions. This study investigated male and female mice separately due to gender factors in periodontitis pathology. All animals were euthanized by the end of the experiment. Their gingival tissue RNA was collected.

Outcome Evaluation:

Proinflammatory cytokine genes, including IL-1b, IL-6, $TNF-\alpha$, and extracellular matrix degradation enzymes such as MMP8 and MMP9 gene expression were examined by real-time RT-PCR.

Results:

2-PCPA treatment significantly reduced inflammatory cytokine gene IL-1b, IL-6, TNF- α , and tissue extracellular matrix degradation enzyme MMP8 and MMP9 gene expression in periodontitis gingival lesions in both early-stage lesions and advanced lesions.

Conclusion:

The local oral administration of the epigenetic drug 2-PCPA could reduce inflammatory tissue breakdown in periodontitis prevention and treatment.

Poster Presentation

Division

Pre-Dental/Undergraduate Student

Abstract Title

Exploring the Impact of TLR2 Signaling on Alveolar Bone Resorption after Ecological Time-sequential Polybacterial Periodontal Infection in the TLR2-/- mice.

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Authors

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Area of Interest

Basic Science Research

Abstract

INTRODUCTION: Periodontitis is a polymicrobial dysbiotic chronic inflammatory disease caused by microbes including bacteria, viruses, and fungi interacting in the host subgingival sulcus/pocket. Streptococcus gordonii, Fusobacterium nucleatum, Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia are common co-colonizers of the supra- and subgingival sulcus in humans. The innate immune system has a broad range of specificity allowing detection of foreign pathogens, and detection of infectious agents relies to a great extent on a family of evolutionarily conserved pattern recognition receptors (PRR), known as the Toll-like receptors (TLRs), which have a crucial role in early host defense against invading pathogens. Among several families of PRR, TLRs are the bestcharacterized. TLR2 and TLR4 are located on the cell surface and are recruited to phagosomes after activation. The purpose of this investigation was to determine alveolar bone resorption (ABR) after ecological time-sequential polybacterial periodontal infection (ETSPPI) with S. gordonii, F. nucleatum, P. gingivalis+T. denticola+T. forsythia in TLR2-/- mice. The aim of this study was to demonstrate the role of TLR2 signaling deficiencies critical in ABR induced by polybacterial periodontal infection. METHODS: Ten-week-old male and female TLR2-/- mice (n=16) were used for polymicrobial ETSPPI infection (Group I) and sham-infection in TLR2-/- mice (Group II). Both maxilla (left and right) and mandible (left and right) were harvested after 17 weeks of ETSPPI infection. The right mandibles and maxilla were autoclaved, and the defleshed jaws were immersed in 3% hydrogen peroxide for 30 min and air-dried. The teeth samples were then stained with methylene blue. Two-dimensional molar teeth images were captured using a stereo dissecting microscope. The area between the cemento-enamel junction (CEJ) to the alveolar bone crest (ABC) of the buccal and the palatal surfaces of the maxillary jaws was measured by using the line tool. RESULTS: TLR2-/- mice exposed to 17-week-polymicrobial infection did not result in significantly enhanced ABR relative to their uninfected control mice in the right mandible lingual, right maxilla buccal, right maxilla palatal, left maxilla buccal and left maxilla palatal sides of the oral cavity. CONCLUSION: The bacteria colonized on the gingival surface in the polybacterial TLR2-/- infected mice

but did not induce enhanced ABR may be due to lack of TLR2 receptor.

Poster Presentation

Division

Pre-Dental/Undergraduate Student

Abstract Title

Using machine learning and non-destructive testing to predict the mechanical properties of custom-fit mouthguards

Presenter' Name:

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Authors

Thomas Duarte, Raafi Zaman, Miles Turner, Dayane Oliveira, Mateus Rocha

Area of Interest

Clinical Science Research

Abstract

Objectives: Develop a machine learning model to predict the mechanical properties (energy absorption, tensile strength, and elastic modulus) based on a non-destructive hardness test. Materials and Methods: Ethylene Vinyl Acetate (EVA) and EVA + polyurethane (PolyShok) laminates were tested at 3- and 4mm thickness (n=12). Shore hardness and thickness were measured at four points using a Shore A hardness tester (Buffalo Inc.). The laminate samples were then cut into dumbbell-shapes with a Type V die cutter, per ASTM-D638 standards. Specimen thickness was measured using an ANSI-certified micrometer (± 0.01 mm accuracy). Samples were tested on an Instron machine (Electro Puls 3000, 1kN loadcell) at 8 mm/min until fracture or 50% strain. Tensile strength, elastic modulus, and energy absorbed were derived from the stress-strain curve. All data were analyzed using a two-way analysis of variance (ANOVA) with level of significance of 0.05 and Tukey's test. A Random Forest Regressor was utilized to predict the properties of energy, modulus, and strength, using the material's hardness as the input variable. The dataset was divided into training and test subsets to train the model and evaluate its predictive accuracy. The Mean Absolute Error (MAE) was determined by computing the average absolute difference between the model's predictions and the actual observed values for energy, modulus, and strength, subsequently converting this error into a percentage of the mean observed values to assess relative accuracy. Results: The average strength (in MPa), modulus (in MPa), energy (in J), and hardness (in Shore A) for EVA were found to be 14.50 (±0.62), 6.52 (±0.43), 25.18 (±5.15), and 80.42 (±0.60) respectively. For Polyshok, these values were 7.57 (±2.11) for strength, 4.79 (±0.31) for modulus, 10.06 (±2.73) for energy, and 75.85 (±1.16) for hardness. ANOVA results showed material type significantly influences all properties (p < 0.001). Thickness had no significant impact, with p-values between 0.132 and 0.724. Tukey's test further confirmed EVA's superiority over Polyshok. The MAE for the model's predictions on hardness to estimate energy, modulus, and strength are 87.9 %, 92.2%, and 94.5%, respectively. Conclusion: Material selection is crucial for athletes' protection with EVA showing superior performance than Polyshok. The machine learning model achieved high accuracy for predicting energy absorption, strength, and modulus, based on a non-destructive hardness test.

Poster Presentation

Division

Pre-Dental/Undergraduate Student

Abstract Title

Cryptococcus neoformans glucuronoxylomannan compromises microglial chemotaxis via inhibition of purinergic receptors

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Authors

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Area of Interest

Basic Science Research

Abstract

Purinergic receptors (PR) are a class of cellular membrane receptors that respond to extracellular purines such as ATP and ADP, which may serve as an alternative mode of activation in immune cells. The ionotropic (P2X) and metabotropic (P2Y) PRs in microglia play a significant role in their activation, proliferation, and chemotaxis. Cryptococcus neoformans is an encapsulated fungus that causes life-threatening meningoencephalitis in individuals with AIDS. Given the limited data on the interactions of the fungus and microglia, we investigated the impact of glucuronoxylomannan (GXM), the main component of C. neoformans capsule, on P2X4 and P2Y12 receptors on microglia and phosphoinositol-3-kinase (PI3K) signaling pathway. We hypothesized that the GXM inhibits microglia's PR surface distribution and P13K signaling. To test this, microglia were pretreated with 10 µg/mL GXM for 4-hours at 37°C in 5% CO2, followed by 2-hours activation with ADP. Confocal microscopy and flow cytometry were used to measure microglial PR distribution upon GXM exposure, while immunoblot was used to assess the expression of PR and the components of the P13K pathway. Inhibitors NP-1815PX (P2X4) and Clopidogrel (P2Y12) were used as negative controls. Bacterial lipopolysaccharide, which activates PR's Pl3K signaling, was used as a positive control. Our results indicate that GXM inhibits the expression of P2X4 and P2Y12, which may have a detrimental role in microglial chemotaxis and infection control due to inactivation of the PI3K signaling cascade. Future experiments will use mouse models of cryptococcal infection to understand the effects of Cn GXM on microglia PR in brain infection control.

Poster Presentation

Division

Pre-Dental/Undergraduate Student

Abstract Title

Understanding the Influence of Psychological Resilience on Patient Well-being during Medical Interventions

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Ben / L / Ofri

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Authors

B. L. Ofri*, T. Vasilopoulos, E. J. Bartley

Area of Interest

Clinical Science Research

Abstract

Background: Chronic low back pain (cLBP) is a leading cause of disability among older adults and is significantly associated with decrements in physical and psychological functioning. As the aging population in the United States continues to expand, the burden of cLBP is on the rise, necessitating urgent attention to geriatric pain management. In recent years, there has been a growing interest in the utility of resilience-based psychological interventions for chronic pain; however, this has been an understudied area in aging adults.

Aims: The aim of this study was to examine the effects of a novel resilience-focused intervention on pain and psychological outcomes in a sample of older adults with cLBP.

Methods: Fifty adults, ages >50 years, with cLBP were recruited for a single-arm trial consisting of 7 weekly telehealth group sessions. The intervention integrated positive psychology concepts (e.g., positive affect, pain acceptance, hopeful thinking, pain self-efficacy) and well-established cognitive-behavioral techniques (e.g., relaxation) for pain management. Key outcome measures include pain severity, pain interference, pain impact, depressive symptoms, and health-related quality of life, assessed at baseline and post-treatment.

Results: Results demonstrated that patients thought the intervention had high credibility (M=7.7/10, SD=1.6) and that they had high engagement (M=6.3/8, SD=1.0) in the intervention. Pain intensity (mean change= -1.2, 95%CI: -1.8,-0.7, p<.001), pain interference (mean change= -3.7, 95%CI: -5.3,-2.2, p<.001), pain impact (mean change= -2.7, 95%CI: -4.1,-1.2, p<.001) and quality of life (mean change= 0.28, 95%CI: 0.01,0.55, p=.04) significantly improved from pre- to post-intervention, while observed reductions in depressive symptoms did not achieve statistical significance (mean change= -1.7, 95%CI: -2.5,0.2, p=.08).

Conclusion: With promising feasibility and efficacy results, the findings from this study provide support for the use of resilience-focused interventions for aging adults with cLBP, which may be a step toward improved pain management in this population.

Poster Presentation

Division

Pre-Dental/Undergraduate Student

Abstract Title

Genetic Characterization of the glucose-PTS in Streptococcus sanguinis, examining competitiveness and fitness.

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Authors

Danniel Pham and Dr. Lin Zeng

Area of Interest

Basic Science Research

Abstract

The oral cavity harbors a diverse microbial ecosystem, where shifts from homeostasis to dysbiosis can lead to oral diseases such as dental caries. Streptococcus sanguinis, a commensal bacterium, is pivotal in maintaining oral health through its metabolic activities, notably influenced by the phosphoenolpyruvate:carbohydrate-phosphotransferase system (PTS). This study aims to elucidate the genetic underpinnings of the glucose-PTS in S. sanguinis and its implications on bacterial competitiveness and fitness within the oral microbiome. By examining single nucleotide polymorphisms (SNPs) in the manL gene and HPr units, we investigate the adaptive mechanisms that confer advantageous traits against cariogenic bacteria, particularly Streptococcus mutans. Employing genetic engineering, bioinformatic analysis, and metabolic assay usage, we characterized mutations in the glucose-PTS's EIIAB (manL) and HPr units within clinical strains of S. sanguinis. Our approach included assessing growth patterns, hydrogen peroxide metabolism, and the bacteria's ability to thrive under complex nutritional conditions. Mutations in the glucose-PTS components, particularly within the manL and HPr genes, correlate with altered growth dynamics and hydrogen peroxide production without significantly impacting acidogenicity. These genetic variations contribute to S. sanguinis's competitiveness in the oral cavity, potentially influencing the microbial landscape toward a healthier equilibrium. Understanding the genetic mechanisms that bolster S. sanguinis's role in oral health presents new avenues for preventive and therapeutic strategies against dental caries. The insights gained pave the way for developing personalized oral care solutions, including diagnostics, probiotic formulations, and genetically engineered microbial interventions to enhance the oral microbiome's resilience against pathogenic colonization.

Poster Presentation

Division

Pre-Dental/Undergraduate Student

Abstract Title

Robust Astrocyte Activation in Response to Cryptococcus neoformans Brain Infection in Mice

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Authors

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Area of Interest

Basic Science Research

Abstract

The encapsulated fungus Cryptococcus neoformans (Cn) is the causative agent of cryptococcosis. Despite aggressive antifungal treatment, immunocompromised patients are highly susceptible to develop and die from cryptococcal meningoencephalitis (CME). The polysaccharide capsule of Cn greatly affects the host immunity and its main component, glucuronoxylomannan (GXM), enhances fungal brain infection. Cn enters the brain via cerebral capillaries and crosses the blood brain barrier (BBB) utilizing diverse mechanisms. However, there are important knowledge gaps regarding underlying brain cell responses and the consequences of this fungal infection for brain function. Astrocytic activation in response to various central nervous system insults is fundamental for brain homeostasis. Reactive astrocytes are associated with destructive cryptococcal brain lesions and high accumulation of GXM in tissue from patients with CME. Therefore, we hypothesized that as Cn colonizes brain tissue during infection, there is a close interaction between Cn and astrocytes that modulates distinct intracellular pathways in both cells. To test this hypothesis, we first used a mouse model of Cn infection and quantified the number of reactive astrocytes using immunohistochemical techniques. Compared to uninfected controls, infected mice had significantly more activated astrocytes surrounding Cn lesions. In addition, using an in vitro model of astrocytic cells exposed to GXM, we observed a significant increase in the expression glutamatergic transporters and receptor subunits. Similarly, we found that the Cn capsule enlarges, and genes involved in capsule formation are increased in Cn cells exposed to astrocytes. We are currently expanding these findings to fully determine the molecular and cellular mechanisms underlying the astrocytic response during CME. Identifying mechanisms by which Cn interacts with astrocytes will provide novel insights into the neurotropism of this deadly infection. This information may also offer new avenues for combating CME, a disease that kills ~200,000 people per year worldwide.

Poster Presentation

Division

Pre-Dental/Undergraduate Student

Abstract Title

Understanding the Correlation between Polymerization Kinetics, Depth of Cure, and Volumetric Shrinkage in Resin-Based Composites

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Area of Interest

Basic Science Research

Abstract

Objectives: To evaluate the association of the polymerization kinetics and the depth of cure (DoC) and volumetric shrinkage (VS) of resin-based composites (RBC).

Methods: Conventional and universal resin composites (3M Filtek Supreme Ultra – FSU; 3M Filtek Universal Restorative – FUR; and, BISCO prototype Universal Composite - BUC) were tested along with bulk-fill resin composites (BISCO Reveal HD Bulk – RHDB; and, 3M Filtek One Bulk Fill -FOBF). DC% was measured at the top (0 mm) and bottom surfaces (2 mm for conventional composites; 2 and 4 mm for bulk-fill composites) by FTIR calculated from aliphatic (1638 cm-1) and aromatic (1610 cm-1) C=C absorption signals. Rp was calculated as the first derivative of DC% when collected at a rate of 1 scan/s during light-curing. VS was measured 10 min after curing by AcuVol. DoC was determined following ISO 4049:2019. All tests were performed on A3 shades at n=3 and light-cured with the Valo Cordless using approximately 20 J/cm2.

Results: Differences in DC% and Rp were statistically analyzed (ANOVA, Tukey, p<0.05) for each composite and each depth. At each depth, RHDB showed higher DC% than the other RBC. For DC%, FOBF did not differ statistically from the three conventional composites at 0 mm, but DC% was lower than BDUC at 2 mm. For both bulk-fill and conventional composites, the RBC with highest DoC showed the highest Rp. Despite observed depth of cure (DoC) when following ISO 4049, deeper increments may be less fully cured. Conversely, higher DoC materials may exhibit higher rates of curing, a risk factor for volumetric shrinkage (VS) and/or shrinkage stress.

Conclusion: The trends in DC% and Rp weakly corresponded to trends in VS and DoC. The hypothesis cannot be fully accepted solely based on this data, but the RBC with highest DoC also had highest DC%, Rp, and VS.

Poster Presentation

Division

Pre-Dental/Undergraduate Student

Abstract Title

Biocompatibility of New Polyurethane-based Polymeric Materials for Sports Mouthguard Fabrication

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Authors

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Area of Interest

Basic Science Research

Abstract

Objectives: The aim of this study was to compare the biocompatibility of new polyurethane-based polymeric materials for sports mouthguard fabrication.

Methods: The new polyurethane-based polymeric materials tested were Elastic 50-A and Flexible 80-A (Formlabs). EVA and PolyshokTM (Bufallo) were used as negative control groups. First, L929 fibroblast cells were seeded in 96-well plates using Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) as the culture medium for 24h at 37oC in an incubator with 5% CO2 and 95% humidity. Then, 12mm diameter, 4mm thick samples of each polymeric material tested (n=5) were immersed in 24-well plates filled with non-supplemented DEMEM for 24h and stored in the incubator at similar settings. After 24h, the culture medium was replaced with either fresh non-supplemented DEMEM (blank; positive control group) or each extract medium from the different polymeric materials (n=5). After 72h, the cell viability was evaluated using the (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) (MTT) method. This procedure was replicated three times per ISO 10993-5. A power analysis was conducted to determine the sample size to provide a power of at least 0.8 at a significance level of α =0.05. Statistical analyses were performed using ANOVA and Tukey's tests.

Results: All polymeric materials caused reduction of cell viability (p < 0.0006; F=12.63) in the following order, PolyshokTM < EVA < Elastic 50-A < Flexible 80-A. However, according to ISO 10993-5, all of them were classified as grade 1, reactivity mild.

Conclusions: The new polyurethane-based polymeric materials seem a biocompatible alternative to substitute conventional polymeric sports mouthguards' materials.

Poster Presentation

Division

Pre-Dental/Undergraduate Student

Abstract Title

Revolutionizing Orthodontic Education, Diagnosis, and Treatment Planning Using Virtual and Augmented Reality Technologies

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Area of Interest

Educational Research

Abstract

The utilization of Virtual Reality (VR) and Augmented Reality (AR) technologies in orthodontics has emerged as an innovative approach to address challenges in illustrating complex biomechanics, enhancing education, improving diagnosis and treatment planning, facilitating patient communication, and enhancing overall treatment outcomes.

Traditional methods of illustrating orthodontic biomechanics have been limited by the lack of 3D graphics, hindering effective visualization and understanding. VR immerses users into computer-generated environments, while AR overlays digital images onto the real world, offering innovative ways to convey complex concepts in orthodontics. These technologies provide interactive and dynamic teaching tools, aiding in education for orthodontic residents and dental students.

In addition to education, VR, and AR play critical roles in diagnosis and treatment planning by bringing three-dimensional images, such as CBCT scans and dental models, to life. Orthodontists can visualize patient records more intuitively and plan treatments with enhanced accuracy. Moreover, patients benefit from VR by visualizing their treatment plans and engaging in virtual consultations, increasing their understanding and commitment to treatment. Patient compliance is also improved through VR applications that provide orthodontic exercises and therapy in a virtual environment, enhancing engagement and adherence to treatment regimens. Integrating VR and AR into orthodontic practice represents a significant advancement, revolutionizing learning approaches, enhancing patient communication, and ultimately improving treatment outcomes.

A comparative study to assess the efficacy of teaching orthodontic biomechanics using VR versus traditional methods such as PowerPoint presentations is underway, highlighting the potential impact of VR on orthodontic education. Furthermore, developing proof of concept systems demonstrates the feasibility and benefits of VR in orthodontic education and practice.

In conclusion, the use of VR and AR technologies in orthodontics signifies a paradigm shift in education and practice, offering immersive, interactive, and engaging experiences that have the potential to transform the field and improve patient care.

Poster Presentation

Division

Pre-Dental/Undergraduate Student

Abstract Title

CRISPR-Cas System can Modulate PGN_1547: a New Hypothetical Virulence Factor in Porphyromonas gingivalis ATCC 33277

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Area of Interest

Basic Science Research

Abstract

Background: Porphyromonas gingivalis is a significant pathogen associated with severe adult periodontitis. It is a part of the dysbiotic biofilm that causes periodontitis, a polymicrobial disease resulting from the coordinated action of a complex microbial community and the host immune response, leading to chronic inflammation and the destruction of tissues supporting the teeth. P. gingivalis can drive the transition of a commensal community to a pathogenic one. However, the fundamental mechanisms underlying this transition related to periodontitis still need to be fully understood. The Clustered Regularly Interspaced Short Palindromic systems-Cas protein-associated system (CRISPR-Cas) is an intricate defense mechanism used by prokaryotes to protect them against invading nucleic acids, including bacteriophages. Emerging evidence points to CRISPR-Cas proteins' involvement in bacterial virulence in various non-oral pathogens. Previously, we showed up-regulation of most CRISPR-associated genes in the oral pathogens P. gingivalis ATCC 33277 and Tannerella forsythia only in periodontitis progressing samples, highlighting its clinical relevance. When it is inside eukaryotic cells, the Cas3 nuclease plays an essential role in controlling the virulence of oral pathogens P. gingivalis.

Objective: In this project, we showed that the CRISPR-Cas system regulates a hypothetical protein PGN_1547 through deletion of Cas 3, a key gene in the Type I-C CRISPR-Cas system in P.gingivalis. This protein contains the DUF2807 domain which belongs to the putative auto-transporter adhesin, head GIN domain family, and (TAAs) superfamily. TAAs are essential virulence factors in Gram-negative pathogens.

Methods: The role of PGN_1547 in pathogenesis was measured using a Galleria mellonella larvae infection model. The biofilm, hemagglutination, and growth curve were performed as previously described.

Results: Deletion of PGN_1547 resulted in a decrease in P. gingivalis' virulence, the ability to form biofilms, and hemagglutination, when compared to the wild-type, without affecting the growth rate. Conclusion: Those results show that the Cas 3 of Type I CRISPR-Cas in P. gingivalis is part of the toolbox that this organism used to modulate the host response and that the hypothetical protein

PGN_1547 is most likely a new adhesin involved in the virulence of P. gingivalis.

Additional Information

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Poster Presentation

Division

Pre-Dental/Undergraduate Student

Abstract Title

Silver-Arginine Nanoparticles for Antimicrobial Properties in Dental Composites

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Area of Interest

Basic Science Research

Abstract

Objective: To evaluate the UV-Vis spectra of spherical and triangular silver nanoparticles (AgNP) stabilized with L-arginine and Poly-L-Arginine.

Materials and methods: Solutions were prepared using 100 mM silver nitrate, 100 mM trisodium citrate, and 0.1 M sodium borohydride. In one flask, 100 μ L of silver nitrate was mixed with 1.5 mL trisodium citrate and diluted with 100 mL water. After adding 1 mL sodium borohydride, the mixture turned yellow within 2 minutes and was stored at 4°C. In another preparation, silver nitrate and trisodium citrate were combined with 280 μ L of 30% hydrogen peroxide, diluted with 100 mL water, and turned from clear to yellow to blue within 30 minutes after adding 1 mL sodium borohydride. After 24 hours, a 0.1 wt% L-Arginine solution and three 0.1 wt% Poly-L-Arginine variations (5-15 Kmol%, 15-70 Kmol%, >70 Kmol%) solutions were formulated and mixed with the AgNPs in addition of 0.033 wt% PVP to prevent aggregation. Centrifugation at 6708 g for 20 minutes was used thrice for purification by replacing the supernatant with fresh water.

Results: The Spherical and Triangular samples with PVP (control) displayed peak absorptions at 394 nm and 800 nm, respectively. When compared to their Poly-L-Arginine counterparts, Spherical AgNP variants showed redshifts ranging from +5 nm to +14 nm, while Triangular AgNP variants exhibited significant blueshifts between -319 nm to -420 nm. These shifts in peak absorption, influenced by the molecular weight of L-Arginine polymers, highlight their impact on nanoparticle optical properties. These molecular red and blue shifts, can be harnessed to tailor surface plasmon resonance, enabling precise control of the surface plasmon resonance for target reactions by dental materials against biofilms.

Conclusions: Spectra analysis of AgNP formulations with different L-Arginine polymers has unveiled noteworthy shifts in UV-Visible peak absorptions that can be used to target reaction in dental biofilms.

Poster Presentation

Division

Pre-Dental/Undergraduate Student

Abstract Title

Periodontal Bacteria Dissemination following Ecological Time-sequential Polybacterial Periodontal Infection (ETSPPI) in the TLR2-/- Mice.

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Area of Interest

Basic Science Research

Abstract

INTRODUCTION: Periodontitis is a polymicrobial dysbiotic chronic inflammatory disease caused by microbes including bacteria, viruses, and fungi interacting in the host subgingival sulcus. Streptococcus gordonii (early bacterial colonizer), Fusobacterium nucleatum (intermediate bacterial colonizer and bridging bacterium), Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia (late bacterial colonizers) are common co-colonizers of the supra- and subgingival sulcus in humans and are considered leading opportunistic bacteria. The innate immune system has a broad range of specificity allowing detection of foreign pathogens, and detection of infectious agents relies to a great extent on a family of evolutionarily conserved pattern recognition receptors (PRR), known as the Toll-like receptors (TLRs), which have a crucial role in early host defense against invading pathogens. The OBJECTIVE of this investigation was to determine intravascular dissemination of multiple oral bacteria including S. gordonii, F. nucleatum, P. gingivalis+T. denticola+T. forsythia during ecological time-sequential polybacterial periodontal infection (ETSPPI) in toll-like receptor knockout (TLR2-/-) mice. METHODS: Ten-week-old male and female TLR2-/- mice (n=16) will be used for polymicrobial infection (Group I) and sham-infection (Group II). The internal organs including heart, lungs, liver, kidney, spleen, and brain were harvested after 17 weeks of infection and stored at -80° C. Genomic DNA extracted from an aliquoted tissue for bacterial genomic DNA detection using 16S rRNA gene-specific polymerase chain reaction (PCR). The amplified DNA visualized using 1.5% agarose gel electrophoresis. RESULTS: S. gordonii, P. gingivalis, T. denticola and T. forsythia gDNA was observed in multiple internal organs of TLR2-/- mice compared to sham-infected TLR2-/- mice. Heart, lungs, and liver are the major organs that were positive for genomic DNA. CONCLUSION: The results strongly suggests that oral bacteria can disseminate intravascularly from oral mucosa to multiple internal organs.



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DMD

ABSTRACT PRESENTATIONS







Poster Presentation

Division

DMD

Abstract Title

Epithelial IFNAR Mediated Regulation of Influenza Control and Lung Inflammation

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Authors

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Area of Interest

Basic Science Research

Abstract

Objectives: Type-I interferons (IFN-I) are crucial to influenza control in the lung. However, the doubleedged role of IFN-I in viral control vs. lung pathology is increasingly appreciated. Alveolar epithelial cells (AECs) are the primary cell target of viral replication in the lung. Paradoxically, while AEC cell death is crucial to viral clearance, overwhelming cell death of AECs is fundamental to lung injury/pneumonia manifestation. To investigate the role of AEC tissue-specific IFN-I signaling in viral clearance vs. tissue damage, we developed a novel conditional mouse strain with AEC-specific deletion of IFN-I receptor (IFNAR) (Ifnar conditional knockout or cKO).

Methods: The deletion of IFNAR signaling in AECs was determined by genotyping and immunofluorescence (IF) based analysis. Lung tissue pathology was determined by H&E tissue staining of lung sections. The quantitation of alveolar type I (ATI) and type II (ATII) was performed by immunofluorescence staining. The viral load was determined by TCID50 assay.

Results: Our data show that the deletion of IFNAR signaling in AECs did not impact weight loss between influenza-infected wild-type and Ifnar cKO mice. Both groups regained the lost body weight at a comparable rate, showing no significant differences in their recovery. Notably, the deletion of IFNAR did not impact viral clearance. On day 7, both groups showed a similar viral load in the lung and cleared the viral load by day 14 post-infection. Further, there was no difference in lung inflammation between wild-type and Ifnar cKO mice at day 7 and 14 post-infection time points. However, despite comparable lung inflammation between the two groups, the lungs from Ifnar cKO mice (compared to WT) exhibited higher quantitative levels of ATI epithelial cells, crucial to lung repair following influenza injury.

Conclusions: These data show that while AEC-IFNAR axis is dispensable to viral control, IFNAR deficiency enhances ATI responses and could lead to expeditious repair of influenza-infected lungs.

Poster Presentation

Division

DMD

Abstract Title

The Role of Wavelength and Radiant Emittance of Curing Lights in Optimizing Light Transmittance and Minimizing Temperature Increase

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Authors

Morgan Evans, Eden Sabag, Mateus Rocha, Dayane Oliveira

Area of Interest

Basic Science Research

Abstract

Objectives: To evaluate the role of wavelength and radiant emittance of curing lights in light transmittance and temperature increase through resin-based materials.

Material and Methods: Light-emitting diodes (LEDs) emitting different spectra (blue, green and red) were designed and characterized using a spectrometer (MARC Resin Calibrator, BlueLight Analytics). Voltage (V) and amperage (A) from each LED were calibrated to emit different radiant emittances tested in this study: 500 mW/cm2, 1000 mW/cm2, 2000 mW/cm2, 3000 mW/cm2 and 4000 mW/cm2. A model self-cured resin-based composite was manipulated in two pastes, one containing benzoyl peroxide and, the other containing ethyl 4-dimethylaminobenzoate. The two pastes were mixed and placed in a mold (d=10 mm, 2 mm thick) until completely cured. Light transmittance through the 2 mm thick resin-based sample was evaluated using the spectrometer (Marc Resin Calibrator, BlueLight Analytics). Temperature increase (oC) in the resin-based sample induced by the exposure to the various spectral irradiances tested were live recorded using an thermal infrared camera (FLIR ONE PRO, FLIR Systems).

Results: The data reveled that light transmission was systematically increased with both radiant emittance and wavelength. Conversely, temperature increase was found to be directly proportional to the radiant emittance, but inversely proportional to wavelength, indicating that longer wavelengths might lead to less heat generation during light curing procedures.

Conclusions: Within the limitations of this study, it was possible to conclude that longer wavelengths seem to be a great alternative to enhance light transmittance through resin-based materials, while mitigating heat generation concerns during light curing procedures.

Poster Presentation

Division

DMD

Abstract Title

Cannabidiol-Oxycodone Interactions with Pain in Rats Measured by Place Conditioning

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Area of Interest

Basic Science Research

Abstract

Pain is a common reason for seeking medical care, and one avenue of treatment is opioid prescription. However, opioids are prone to misuse, and deaths linked to opioid involvement are a major concern. This has led to a call for safer, more efficacious alternatives. Our previous studies suggest that the marijuana derivative cannabidiol (CBD) augments the analgesic effect of the opioid oxycodone. The purpose of this study was to evaluate the ability of CBD to potentiate the affective pain-relieving effects of oxycodone using the conditioned place preference paradigm in rats experiencing chronic pain. Sprague Dawley rats underwent a chronic constriction injury (CCI) of the sciatic nerve or sham surgery. The efficacy of the CCI surgery was confirmed using a cutaneous thermal reflex pain assay. Rats were then conditioned either to CBD (10 mg/kg), oxycodone (0.56 mg/kg), or a combination of both. Tests of conditioned place preference were administered the day after conditioning and again 7, 11, and 14 days later. CCI surgery profoundly reduced paw withdrawal latency in the surgeried leg indicative of a neuropathic pain state. The combination of CBD and oxycodone produced a stronger conditioned place preference irrespective of surgery status relative to the two drugs alone. CBD may potentiate the ability of oxycodone to relieve a pain state induced by both CCI or sham surgery. These studies warrant further investigation of the potential utility of CBD as an adjunct to opioids for treating pain.

Poster Presentation

Division

DMD

Abstract Title

Artificial Intelligence for Prediction of Location and Severity of Impacted Teeth

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Area of Interest

Clinical Science Research

Abstract

Objectives:

1) Use of AI model for automatic localization and assessment of prognosis of impacted teeth will have improved predictability of the orthodontic treatment.

2) To create an algorithm for identification and characterization of maxillary impacted teeth in panoramic radiographs by using R-CNN

3) Evaluate accuracy and reliability of the AI model as well as correlate its output to that of clinicians with varied clinical experience

Methods:

We will use a heterogenous dataset of more than 5000 panoramic radiographs collected from the UFCD Department of Oral and Maxillofacial Radiology to develop & train the AI model. As the model accuracy is highly dependent on the quality and quantity of the data set, preprocessing of the panoramic radiographs will be performed before it is fed into the AI model. Specifically, 1) pixel rescaling will be employed so the pixels contribute evenly to the learning process in the network, 2) resizing of the images will occur to ensure all images are the same size, and 3) augmentation and image resolution will occur to increase the number of pixels and thus increase the size of data set. Then, the model will be constructed using R-CNN, a deep learning algorithm. R-CNN will segment the maxillary teeth to identify each tooth in maxillary arch including impacted teeth. Following the identification of impacted teeth and their location, classification for severity of impaction based on clinical standards will be incorporated into the algorithm. The reliability and accuracy of the AI model developed will be evaluated using an independent validated data set of 1000 panoramic radiographs from their own practice, we will compare the output of the AI

model with the interpretations made by clinicians with varying levels of experience.

Results: We are currently creating the segmentations for the AI system and will have a large enough data set complete to present results at the meeting.

Conclusions: This project will establish a systematic, cost efficient and safe approach for diagnosing impacted teeth based on the commonly captured panoramic X-rays with minimal lonized Radiation exposure. As a result, the predictability, consensus and communication between dental professionals on prognosis of impacted teeth will be improved. An intuitive user-friendly interface (UI/UX) for clinical application of the R-CNN based technology with also

Poster Presentation

Division

DMD

Abstract Title

Genetic Characterization of Glyoxalase Pathway in Oral Streptococci

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Area of Interest

Basic Science Research

Abstract

Objectives: Methylglyoxal (MG) is a highly reactive byproduct of glycolysis. For detoxifying MG, caries pathogen Streptococcus mutans (SMU) and oral commensals such as Streptococcus gordonii (SGO) harbor lguL and related genes of the glyoxalase pathway. SMU and SGO have an antagonistic relationship, with SGO being associated with oral health and SMU with increased risk for caries. Here we analyzed the glyoxalase pathway in both streptococci to understand the role of MG in their relationship.

Methods: Mutants deficient in lguL, an uncharacterized paralog of lguL (gloA2), and several related genes, were constructed in SMU and SGO via allelic exchange. All wild type and mutants were subjected to MIC assays for MG tolerance, morphological profiling, growth analysis, eDNA measurement, and qRT-PCR for metabolically relevant genes upon MG exposure.

Results: MIC profiling indicated that SMU was significantly more resistant to MG than most commensal streptococci, which contributed to its competitive fitness. Deletion of lguL in SMU resulted in a far more severe reduction in MG tolerance than a similar deletion in SGO, with both mutants, Δ SMU.1603 and Δ SGO.1035, now showing comparable MICs for MG. The mutants of gloA2 showed little change in MIC levels, however a drastic growth deficiency in both species when growing on fructose, including chaining and release of eDNA. Treatment with MG increased mRNA levels of lguL in both species, but not gloA2. Expression of SMU.1602 (a redox protein) and SMU.1605 (an efflux pump) was not affected by MG, however, SMU.1605, and SMU.1602&1603 to a lesser extent, appeared to be negatively regulated by SMU.1604 (padR) independent of MG exposure. SGO harbors no orthologs of SMU.1602-1605 other than lguL.

Conclusion: Our comparative genetic analysis suggested that metabolism of MG is regulated by different mechanisms in SMU and commensals, and the contribution of gloA2 to bacterial fitness remains to be clarified.

Poster Presentation

Division

DMD

Abstract Title

Understanding Cryptococcus neoformans Capsular Gene Expression During Interactions with Microglia

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Area of Interest

Basic Science Research

Abstract

ABSTRACT:

Cryptococcal meningitis is a fungal disease affecting immunocompromised individuals, killing ~20% of AIDS patients globally. Its causative agent, Cryptococcus neoformans (Cn) invasion begins by inhalation of the spores, disseminates hematogenously, and reaches the brain by crossing the blood-brain barrier. Cn has a capsule—a main virulence factor—that protects the fungus from phagocytosis by microglia (MG), the main brain immune cells. MG's influence on capsular gene expression of Cn upon interactions is unknown. Therefore, we hypothesized that upon MG activation, Cn will show capsular gene upregulation during their interactions.

OBJECTIVE:

We analyzed the expression of Cn capsular-related genes (cap10, cap59, cas33, grasp) after incubation with MG.

METHODS:

Cn was exposed to naïve or LPS/IFN-γ-activated murine NR-9460 MG (direct contact or indirect exposure) or their derived molecules (supernatant or lysed cells) for 24h at 37°C and 5% CO2. Relative Cn capsular gene expression was quantified by qPCR. GAPDH was used as a housekeeping gene control.

RESULTS:

Cn directly in-contact or indirectly exposed (via a trans-well only allowing chemical exchange) to activated MG and naïve MG, respectively, showed no significant changes in capsular-related genes. Upon Cn exposure to MG supernatant, we surprisingly found that only cap10 was significantly downregulated in activated MG but not in naïve MG. Lastly, Cn exposed to supernatant or lysed activated or naïve MG had no significant changes in gene expression related to capsule production and secretion. Although our results don't support our hypothesis or previous observations, further studies are needed to validate our findings.

CONCLUSION:

The significant downregulation of cap10 following activated microglia supernatant exposure indicates that Cn's capsule can be modulated during these interactions. Understanding the interactions of Cn may help us develop novel treatments and therapeutics for the management of cryptococcal meningitis. This research was supported by NIAID award AI145559.

Poster Presentation

Division

DMD

Abstract Title

Investigating the Role of Pg Sphingolipids on Macrophage Antigen Presentation

Presenter' Name:

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Authors

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Area of Interest

Basic Science Research

Abstract

Objectives: Periodontal disease (PD) is a chronic inflammatory disease initiated by a dysbiotic subgingival microbiota. If untreated, PD can lead to irreversible destruction of the alveolar bone and soft tissues that support the teeth. Recently it was identified that Porphyromonas gingivalis (Pg) can synthesize sphingolipids (SLs). Work from our lab suggests Pg SLs reduce inflammation elicited by this organism and are transferred to host cells and can influence oral bone loss. Preliminary ELISA data using IgG serum collected from Pg W83 WT and SL-null mutant (SPT) challenged mice suggests Pg SLs may influence adaptive immunity. Macrophages play a role in Pg-elicited alveolar bone loss in mice and are professional antigen-presenting cells (APCs) thus potentially providing the bridge between innate and adaptive immunity. It is known that CD1d on APCs can present lipid antigens (i.e bacterial SLs) to T cells. Currently, it is unknown if Pg SLs influences CD1d and other known antigen presenting receptors (i.e. MHC-I and MHC-II). The goal of this study is to investigate the role of Pg SLs on antigen presentation receptor expression via macrophages.

Methods: PMA treatment induced differentiation in the THP-1 monocyte cells to become a macrophage-like cell line. The treatment groups include untreated and Pg W83 WT or SPT (MOI 100). Cells were challenged for 30 minutes, 1 hour, 2 hours, and 4 hours. Using a Qiagen RNeasy RNA Extraction Kit, RNA was isolated, purified, quantified, and transformed into cDNA using Applied Biosystems High-Capacity RNA to cDNA kit. Selected primers were CD1d, MHCI, MHCII, CD80, and CD86. Taq-man qPCR kit was used to analyze gene expression. Gene expression was normalized to housekeeping gene B-actin.

Results: We are currently performing experiments examining target gene expression patterns and preliminary data support that there may be modest elevation in CD1d gene expression in THP-1 cells cultured with Pg Δ SPT mutant compared to WT.

Conclusion: Our preliminary data suggests continued investigation of antigen presentation receptors to determine if the SLs of Pg influence their expression patterns. Any observed changes in gene expression will be confirmed in the future at the protein level as well as T cell activation/proliferation studies. A better understanding of adaptive immunity by Pg SLs furthers our understanding of how these lipids impact host-pathogen interaction and may shed light on periodontal disease pathogenesis.

Poster Presentation

Division

DMD

Abstract Title

Effect of Short- and Long-wavelengths on Physical and Chemical Properties of Light-cured Resin Cements

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Area of Interest

Basic Science Research

Abstract

Objectives: To evaluate the effect of short- and long-wavelengths on physical and chemical properties of light-cured resin cements.

Methods: A multiwavelength-activated photoinitiator was synthesized, and its spectral and molar absorbance were characterized. A model resin-based cement was formulated containing the alternative photoinitiator. The same formulation using camphorquinone was used as a control group. Light-emitting diodes (LED) emitting the wavelengths absorbed by the photoinitiator were developed and characterized. Voltage and amperage were set up for each LED to emit 1000mW/cm2. Different clinical scenarios were tested using lithium disilicate ceramics of three medium translucence shades (A1, A2, and A3) in different thicknesses (1mm, 2mm, and 3mm). The time of exposure was performed according to the manufacturer's instructions, 20s per mm of ceramic. Light transmittance (mW/cm2) and radiant exposure (J/cm2) were evaluated using a spectrophotometer. FT-IR spectroscopy was used to evaluate the degree of conversion of the resin cements. Power analysis was conducted to determine sample size and power of 0.8 at a significance level of α =0.05. Data normality and homoscedasticity were performed using Shapiro-Wilk and Lavene's tests. Statistical analyses were performed with ANOVA and Tukey's test for multiple comparisons.

Results: The alternative photoinitiator absorbed light into the blue and green spectra with a molar absorbance coefficient of 144m2/mol. The LEDs developed demonstrated peaks of emission into 460nm (blue light spectra) and into 520nm (green light spectra), respectively. The alternative photoinitiator showed similar cure efficiency to camphorquinone when exposed to the same testing conditions (p<0.001). Still, although green light demonstrated higher light transmittance and radiant exposure in-depth than blue light (p<0.001), it indicated a 5% lower degree of conversion in-depth.

Conclusion: The higher light transmittance from longer wavelengths doesn't seem to fully compensate for the lower energy from these wavelengths. Still, green light provided clinically acceptable cure efficiency in all clinical

scenarios tested.

Keywords: dental photoinitiators; resin cements; dental curing lights.

Poster Presentation

Division

DMD

Abstract Title

Focus on Access to Care in the Journal of the American Dental Association

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Area of Interest

Clinical Science Research

Abstract

Over 60 million Americans live in Dental Health Professional Shortage Areas; 43% of U.S. dentists accept Medicaid or CHIP making access to dental care a contributing factor in oral health disparities. As the preeminent dental journal, The Journal of the American Dental Association (JADA) has influenced the dental profession since 1913. This study reviews JADA's attention to oral health disparities via trends in socio-economic and geographical contexts over time.

A journal review of JADA titles from January 1913 – August 2020 identified themes relevant to oral health disparities, i.e., the distribution and shortage of dentists, and private/public dentistry trends.

The review identified public health dentistry as the focus of JADA through the first few decades of publication. By the 1950s emphasis shifted to maximizing practice profit with increased promotion of private over public health dental practice. Overall, in the 20th century, articles focusing on serving economically disadvantaged populations were infrequent. In more recent publications, emphasis on disadvantaged populations has steadily increased. For the first 90 years JADA's attention to urban/rural geographic practice maldistribution was sparse; over the past 20 years this attention has increased. Tracking JADA's focus over time aids in assessing the exposure of dental professionals to the sometimes differing oral health needs of the public. This understanding can increase the relevance of informational content for both private and public dental professionals; diversify educational offerings and efforts targeting dental professionals; and elucidate issues critical to improving increased access to oral healthcare.

Poster Presentation

Division

DMD

Abstract Title

Macrophage Activation Impacts Responses to Porphyromonas gingivalis and its OMVs.

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Area of Interest

Basic Science Research

Abstract

Objectives:

Periodontal disease is a chronic oral inflammatory disease of the oral cavity. Porphyromonas gingivalis is linked to this disease and produces outer membrane vesicles (OMVs). Macrophages infiltrate periodontal tissues during disease and exist at these sites in different activation states; however, it is incompletely understood how macrophage activation impacts the host response to P. gingivalis. Sphingolipids (SLs) produced by P. gingivalis present on the bacterium and its OMVs modulate the host's inflammatory response. The objective of this study was to begin to characterize the response of macrophages poised in different activation states to wild type (WT) and SL-deficient P. gingivalis and its OMV.

Experimental Methods:

Human macrophage-like THP-1 cells were treated for 24 hrs. to drive activation to classically activated (M1; TNF- α and LPS) and alternatively activated (M2; IL-4) states. Untreated THP-1 cells served as the non-activated group (M0). Activated macrophages were exposed to WT P. gingivalis and its SL-deficient mutant (MOI 100) or OMVs purified from these strains (1000 particles/cell) and samples were collected temporally up to 24 hrs. Luminex-based immunoassays measured levels of cytokine production (TNF- α , IL-6, IL-8, IL-10, and CCL5/RANTES).

Results:

Luminex results showed a trend for increased cytokine production for M1 macrophages compared to M2, M0 macrophages for both WT and SL-deficient mutant P. gingivalis. Luminex results also showed a trend for increased cytokine production for the SL-deficient mutant in comparison to the WT. These patterns were also observed with OMVs from these strains.

Conclusion:

Our preliminary data support that macrophages poised in different activation states respond differently to P. gingivalis and its OMVs. It is also shown that there is a difference in the cytokine production responses to WT versus the SL-deficient mutant P. gingivalis. Further testing is needed to understand how these data may impact the

progression of periodontal disease.

Poster Presentation

Division

DMD

Abstract Title

Cannabidiol-Oxycodone Interactions on Cutaneous Thermal Reflex Nociception and Reward

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Area of Interest

Basic Science Research

Abstract

Seeking medical care for pain is common, and one approach to treatment involves prescribing opioids. Nevertheless, opioids carry a risk of misuse, and fatalities associated with opioid use are a significant concern. This has prompted a demand for safer and more effective alternatives. Our earlier research indicates that cannabidiol (CBD), a compound derived from cannabis, enhances oxycodone analgesia in an operant orofacial thermal cutaneous pain assay. This study assessed interactions of CBD with oxycodone on thermal cutaneous reflex pain and reward.

In the first study, Sprague Dawley rats underwent either chronic constriction injury (CCI) of the sciatic nerve or sham surgery. Subsequently, they received intraperitoneal treatment with CBD (10 mg/kg), oxycodone (0.56 mg/kg), or both combined. A radiant heat source was applied to the hind paw, and the latency to withdraw the paw was measured. In the second study, rats without any surgery were conditioned to the same drug treatments using a conditioned place preference paradigm.

In the first study, CCI surgery significantly reduced paw withdrawal latency in the surgeried leg, indicating a neuropathic pain state. Averaged across both paws, CBD non-significantly reduced (14%), oxycodone non-significantly enhanced (14%), and the combination significantly enhanced (39%, p < 0.001) paw withdrawal latencies. However, there was no statistical difference between oxycodone and the combination. These effects were not impacted by surgery status and together suggest CBD slightly potentiates the analgesic efficacy of oxycodone in this assay. In the second study, CBD did not produce a conditioned place preference, whereas oxycodone and the combination induced similar-sized conditioned place preferences, indicating that CBD did not enhance oxycodone reward.

Under these experimental conditions, CBD marginally enhances oxycodone analgesia without affecting oxycodone reward. These findings advocate for further exploration of the usefulness of CBD as a supplement to opioids in treating pain.

Poster Presentation

Division

DMD

Abstract Title

Extrusion spring to disimpact impacted maxillary second molars

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Area of Interest

Clinical Science Research

Abstract

Impacted second molars are frequently diagnosed as a secondary finding in an orthodontic clinic. Their prevalence is very low and ranges between 0%–2.3%. [1] Knowledge about their diagnosis, prognosis, and treatment is derived primarily from case reports and very few clinical studies. [2, 3]However, most of these clinical studies and reports are focused on the impacted mandibular second molars. [2, 4]Their treatment is indicated to avoid the risk of resorption of adjacent teeth, caries, periodontal problems, development of cysts, or to help correct the malocclusion. [5] Because of their restricted access, a multidisciplinary treatment approach that involves surgical exposure and orthodontic alignment is required. The challenge is often related to finding a predictable biomechanical approach for their traction and alignment into the arch. This pearl presents a simple and feasible technique for their traction into the arch with the use of an extrusion spring.

Technique

Following the surgical exposure of an impacted maxillary second molar and the insertion of a rigid rectangular working archwire in the upper arch 19*25 SS, an extrusion auxiliary spring can be fabricated with the use of a 17X25" CNA wire. The spring can be inserted in the auxiliary maxillary first molar tube mesially and cinched distal to the first molar as illustrated in figure 1. The activation of this spring is often achieved by opening the loop and pulling its arm downward. Afterward, the active arm is tied to the chain of the surgically exposed impacted maxillary molar (Figure 1). The insertion of a rigid 19X25 SS archwire in the arch before using the spring will prevent the undesired side effects of using the spring on the maxillary first molar (intrusion, rotation, and buccal tipping of its crown).

In our example (figures 2-5), the panoramic radiograph and clinical image for a 15-year-old patient, illustrate the severely impacted maxillary left second molar (figures 2 and 3), with the use of the extrusion spring, the molar erupted into its final position in 4 months following the activation of the spring (figure 4). The spring was removed and a molar tube was placed to align the maxillary second molar into its final position (figure 5).

Poster Presentation

Division

DMD

Abstract Title

Developing a Machine Learning Model for Skeletal Maturity Assessment.

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Area of Interest

Clinical Science Research

Abstract

Objective:

This research aims to create a machine-learning model for automating the classification of Cervical Vertebral Maturation (CVM) stages, assisting orthodontists in treatment planning based on patients' skeletal maturity.

Method:

Cephalometric images were obtained from the AAOF craniofacial growth legacy collection and filtered to remove the low-quality images. Two dental students were trained to identify CVM stages. They labeled 746 images independently using the method described by McNamara and Franchi (2018). An experienced orthodontist verified the staging. Images were cropped to 1800x1800 pixels, focusing on the cervical vertebra, and resized to 224x224 for consistency. After preprocessing (removing duplicates, removing 3-channel images, removing multi-labeled images), we obtained 924 1-channel (grayscale) images. The images were then input into a deep neural network model, ResNet-34, and trained with a batch size of 2 for 150 epochs. The Optimizer was Adaptive Moment Estimation (Adam) with an initial learning rate of 0.0001. A multistep learning rate scheduler was applied, reducing the learning rate by multiplying it with a factor of 0.1 at epochs 75 and 127. The training set consisted of 864 images. The sample sizes for each stage, CS1 through CS6, were 151, 176, 131, 138, 151, and 117, respectively. Randomly selected samples of 10 images from each class, which led to a validation set of size 60.

Results:

The model achieved a classification accuracy of 76.67%. The precision, which is the proportion of true positive results, was 87.5%, 64.28%, 66.66%, 100%, 69.23%, and 90% for CS1, CS2, CS3, CS4, CS5, and CS6 stages, respectively. The sensitivity, which measures how good the test is at identifying stages, revealed that the CS2, CS5, and CS6 stages were 90%, whereas CS1 showed a sensitivity of 70%. This program identified the CS3 and CS4 stages with a sensitivity of 60%.

Conclusions:

In this research, we successfully developed a machine learning model using ResNet-34 to automate Cervical Vertebral Maturation (CVM) stage classification, aiding orthodontists in treatment planning based on skeletal maturity. Achieving an accuracy of 76.67%, the model demonstrated promising precision and sensitivity rates across different CVM stages, with particularly high precision observed for CS4 and CS6. Further validation on larger datasets is needed to enhance its clinical applicability.

Poster Presentation

Division

DMD

Abstract Title

Oral Keratinocyte Extracellular Vesicles Immunomodulate Periodontal Ligament Fibroblasts

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Area of Interest

Basic Science Research

Abstract

It is well accepted that Extracellular Vesicles (EV) are essential for cell-to-cell communication as they carry various cargo in the form of proteins, lipids, and nucleic acids. Thus, EVs play a central role in directing immune responses including the inflammation that occurs in periodontal diseases. Periodontal diseases are a result of chronic inflammation that causes the destruction of the soft and hard tissues surrounding teeth. Oral keratinocytes (HOK) and periodontal ligament fibroblasts (HPLF) are two of the major cell types within the periodontal pocket that are affected and the communication between these two cell types can partly drive the pathophysiology of periodontal disease. The aim of this study was to characterize the EVs from HOK under homeostatic and inflammatory conditions and their effect on HPLF immune function. To achieve this, EVs from HOKs were isolated by differential centrifugation in the presence and absences of LPS to mimic healthy and diseased conditions. The EV sizes and concentrations were then characterized by Nanoparticle Tracking Analysis (NTA). HPLF were exposed to titrating amounts of the resultant EVs for 24 hours and multiplex analysis was used to quantify immune related soluble mediators and mRNA. LPS treatment of HOK did not alter the concentration or size of EVs released into the environment. On the other hand, EVs from LPS stimulated HOK promoted the expression of inflammatory cytokines by HPLF while inhibiting the expression of those associated with homeostasis. EVs from LPS stimulated HOK also promoted the upregulation of proinflammatory genes/pathways in HPLF while inhibiting the induction of metabolic genes/pathways. EVs from HOKs in the absence of LPS had no effect on HPLF soluble mediator or mRNA expression. In conclusion, data here suggests that HOK EV cargo may be different under conditions of health and disease and most likely play a role in promoting periodontal inflammation.

Additional Information

N/A

Poster Presentation

Division

DMD

Abstract Title

Comparative assessment of the efficacy and efficiency of the Invisalign® system compared to the fixed appliance in class II malocclusion

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Area of Interest

Clinical Science Research

Abstract

Objective

This retrospective study compared treatment outcomes and duration between Invisalign® and fixed appliance in adolescent patients with class II malocclusion.

Materials and Methods

Records of 31 Invisalign® and 35 fixed class II patients, aged (13.5±1.6) years were compared. The Discrepancy Index (DI) was used to score initial complexity. The weighted total and individual PAR index scores were used to compare clinical outcomes. Data on demographics, treatment and finishing durations, number of visits and refinements, duration of class II correction, and post-treatment IMPA angle were collected.

Results

The DI scores for Invisalign® and fixed appliance groups were comparable (P>0.05). The mean pre-, post-treatment, and overall reduction in weighted PAR scores between the groups were not significantly different (P>0.05). The Invisalign® group had a significantly shorter treatment duration (20.0 ± 11.6 months) than the fixed group (27.4 ± 9.1 months) (P<0.001). The number of visits for the Invisalign® group was significantly less (12.7 ± 6.2) than those in the fixed group (17.8 ± 5.8) (P<0.001). Duration for class II correction was significantly shorter for Invisalign® (13.3 ± 10.0) compared to the fixed group (17.4 ± 9.0 months) (P=0.026). A smaller post-treatment change in the IMPA ($2 \pm 6^{\circ}$) was detected in the Invisalign® group compared to the fixed group ($5 \pm 6^{\circ}$) (P<0.05).

Conclusions

Treatment outcomes for Invisalign® in class II adolescent patients were comparable to those achieved in fixed appliance. Shorter treatment duration, class II correction duration, fewer visits, and better control for the IMPA were noticed in the Invisalign® group.

Keywords: Invisalign®, Fixed appliance, Class II malocclusion, Class II elastics

Poster Presentation

Division

DMD

Abstract Title

Effect of short- and long-wavelengths on physical and biological properties of light-cured resin restorations

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Area of Interest

Basic Science Research

Abstract

Objectives: This study aimed to evaluate the effect of short- and long- wavelengths on the physical and biological properties of light-cured tooth restorations.

Methods: A multiwavelength-activated photoinitiator was synthesized, and its spectral and molar absorbance were characterized using spectrophotometry. A model bulkfill resin-based composite was fabricated containing the photoinitiator, ethyl 4-dimethylaminobenzoate and diphenyliodonium hexafluorphosphate. Light-emitting diodes (LED) emitting different spectra (blue, 460nm and red, 620nm) were developed and characterized using a spectrophotometer. Voltage (V) and amperage (A) were set up for each LED to emit an irradiance of 1000 mW/cm2. Class I preparations 5 mm long, 4 mm wide, and 4 mm deep were performed in extracted third molars, leaving 0.5 mm of dentin at the pulpal floor. Uncured bulk fill increments were placed in the teeth mold. Light transmittance through the restorations while exposed to different wavelengths was evaluated using the spectrometer. Raman spectroscopy was used to evaluate cure efficiency. Temperature increase (oC) at the pulpal floor was live recorded using an infrared camera and a customized oral cavity chamber simulator to standardize the initial temperature at 36oC. Cell viability was evaluated using the MTT method.

Results: The multiwavelength-activated photoinitiator absorbed light into the blue and red spectra with a molar absorbance of 144. Red light showed higher light transmittance in-depth than blue light (p<0.001). Blue and red light provided a similar degree of conversion (p>0.05). The red light did not cause a temperature rise, while blue light was capable of increasing temperature over 5.5oC in 3 seconds (p<0.001). Blue light caused overall reduced cell viability (p<0.001).

Conclusions: The use of longer wavelengths seems a viable, harmless alternative to substitute conventional short wavelengths used in Restorative Dentistry.

Poster Presentation

Division

DMD

Abstract Title

Characterization of Warm Temperature Effects on TRPM8 Knockout Mice

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Area of Interest

Basic Science Research

Abstract

Objectives: Colder temperatures are thought to be detected by the Transient Receptor Potential Cation Channel, Subfamily M, Member 8 (TRPM8) expressed in primary afferent fibers. Though TRPM8 has been well-characterized in cold sensitivity, further investigation into TRPM8's impact on warm thermoception is not complete. This study addresses the characterization of TRPM8's role in warm temperature perception.

Methods: Data are collected using electrophysiological and behavioral assays. For electrophysiological classification, trigeminal ganglia neurons (TRG) are patched in a whole-cell voltage-clamp configuration using channel classification protocols for HCN, voltage-gated potassium, and voltage-gated sodium channels. The shuttle box assay utilizes nine equally spaced 2.5" zones, which mice move freely throughout. Trials are recorded using a digital camera and scored by two investigators. The Orofacial Pain Assessment Device (OPAD) is a risk/reward-based assay that characterizes temperature preference, sensitivity, and comfortability in hairless wild-type and knockout mice. Mice are tested at randomized temperatures to prevent the animals from learning the sequence. Both behavioral assays utilize 10-minute trial durations.

Results: Patch-clamp data indicates knockout TRG cells display greater inward currents from HCN channels and greater outward currents from voltage-gated potassium channels. Evaluation of shuttle box assay data indicates that wild-type mice spend more time in warmer temperature zones while knockout mice spend more time in colder temperature zones. In OPAD, the knockouts were reluctant to contact the stimulus when >30°C, while wildtype mice find 30-40°C the most comfortable temperature range.

Conclusion: Physiologically, TRG neurons display significant differences in HCN and voltage-gated potassium channels, suggesting a differential in signaling patterns due to a lack of TRPM8 expression. Behaviorally, shuttle box data indicates wild-type mice hold a preference for warmer temperatures while knockout mice prefer colder temperatures. OPAD data corroborates such findings, with reluctance of

knockout mice to contact Peltier pads when stimulus reaches >30°C.

Poster Presentation

Division

DMD

Abstract Title

Surface-associated Cyclic-di-AMP Phosphodiesterase Enhances Virulency of Enterococcus faecalis

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Area of Interest

Basic Science Research

Abstract

Objectives:

Cyclic-di-AMP is a bacterial signaling nucleotide essential for both homeostatic and pathogenic mechanisms, such as osmoregulation, antibiotic tolerance, and biofilm formation. Further, cyclic-di-AMP is crucial in host-pathogen interactions due to potent activation of the cGAS-STING-mediated type I interferon (IFN) pathway in the host when released extracellularly. Prior studies indicate that in E. faecalis, the diadenylate cyclase (DAC) cdaA and the intracellular phosphodiesterases (PDEs) dhhP and gdpP are the sole synthesizing and degrading peptides of cyclic-di-AMP, respectively. Recently, a surface-associated, extracellular PDE unique to E. faecalis (OG1RF_10056) was discovered. The purpose of this study is to investigate the role of OG1RF_10056 in triggering host immune responses, and how its inactivation augments these responses.

Methods:

RAW 267.4 macrophage cells were infected with one of two E. faecalis strains: OG1RF (parental strain) or Δ OG1RF_10056 (generated by markerless deletion) at a multiplicity of infection of 20. Intracellular bacterial survival within infected RAW 267.4 cells was assessed at 0-, 2-, 4-, and 24-hours. qRT-PCR was used to quantify mRNA levels of IFN- β 1 and TNF- α over time, which were normalized to GAPDH expression levels. Uninfected cells were used as controls.

Results:

 $\Delta OG1RF_{10056}$ exhibited higher intracellular macrophage survival when compared to the OG1RF strain. qRT-PCR analysis indicated $\Delta OG1RF_{10056}$ -infected macrophages expressed higher IFN- β 1 levels when compared to macrophages infected with the parent strain. However, $\Delta OG1RF_{10056}$ -infected macrophages differed in the expression of TNF- α , which is not known to be activated via the cGAS-STING pathway.

Conclusion:

Preliminary findings suggest increased accumulation of extracellular cyclic-di-AMP, as seen in Δ OG1RF_10056, results in activation of the cGAS-STING pathway and, paradoxically, enhanced persistence of E. faecalis within RAW 267.4 macrophages. The differences observed in TNF- α expression suggest extracellular cyclic-di-AMP may

Poster Presentation

Division

DMD

Abstract Title

Evaluation of Sports Mouthguards Wear in Hockey Student Athletes: A Clinical Prospective Study

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Area of Interest

Clinical Science Research

Abstract

Purpose: The purpose of this study was to evaluate the clinical wear of sports mouthguards in student athletes after 3 months of use.

Materials and Methods: The study was approved by IRB (#202300068). All recruited participants were submitted to a clinical exam to exclude any participants with dental care needs prior to the study. Then, all eligible subjects (n=12) were submitted to impressions with polyvinylsiloxane (PVS) for the fabrication of their custom-designed sports mouthguard. All custom-designed sports mouthguards were fabricated using ethyl-vinyl acetate thermoplastic material (PolyShok, Buffalo Inc.), 4 mm of thickness, using the vacuum technique, and the mouthguard was delivered to the subject after thickness and correct occlusion points were checked prior to delivery to be used during practice and games. The follow-up visit corresponded to a 3-month follow-up where the amount of wear was evaluated by the thickness that was measured using a micrometer in six standardized spots: the incisal edge of each tooth from canine to canine. The data was submitted to a two-way repeated measures analysis of variance (ANOVA) with level of significance of 5 %.

Results: Statistical analysis did not reveal significant changes in mouthguard thickness between baseline (Time 0) and three months later (Time 3), with p-values ranging from 0.277 to 0.996.

Conclusions: This study emphasizes the critical need for personalized mouthguard fittings and consistent evaluations of their thickness for optimal protection at every tooth location. It suggests the necessity for long-term investigation and the creation of standards for more resilient and flexible mouthguard designs to enhance or sustain minimum protection over time.



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MS/RESIDENT

ABSTRACT PRESENTATIONS







Poster Presentation

Division

MS/Resident

Abstract Title

A Dilemma in the Dental Curriculum: Obstacles and Concerns When Removing Caries

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Area of Interest

Educational Research

Abstract

Introduction: Removal of caries is a crucial step for maintaining the health of a tooth. With the new concept of dental remineralization, clinicians face a clinical dilemma of the best way to proceed, leading to an error in their clinical decision to remove the carious tissue and perform indirect or direct pulp capping on permanent teeth.

Objectives: The purpose of this study was to retrospectively assess the pulp capping procedures performed at our institution and make available the information to calibrate the faculty so that the protocol for these procedures can be revised.

Methods: The study included patients who were treated in the predoctoral clinics with either a direct or indirect pulp capping procedure from June 2019 to September 2023. Charts of 345 subjects were identified. Data collected encompassed patient demographics (age, sex), type of capping agent, vitality testing for diagnosis at the time of the procedure and whether recall was done with radiographs. Results: Of the 276 teeth that had an indirect pulp capping procedure, 101 teeth (36.59%) had no vitality test performed at the time of the visit. Follow-up occurred in 130 (47.20%) teeth, mostly with bitewing radiographs. Of the 69 teeth that had direct pulp capping, 30 (43.47%) were not tested, and 37 teeth (53.62%) had a follow up done.

Conclusion: Through our data analysis, we concluded that the protocol for these procedures should be revisited among the dental school faculty and perhaps coded and flagged by Axium, so that proper treatment approach is utilized.

Poster Presentation

Division

MS/Resident

Abstract Title

Sebaceous Carcinoma with Involvement of the Oral Cavity: A Case Report of a Rare Occurrence

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Area of Interest

Clinical Science Research

Abstract

Introduction: Sebaceous carcinoma (SC) is an aggressive, rare cutaneous malignancy. It shows striking predilection for the skin of the eyelid, face, neck, and scalp, but may arise on any cutaneous site. Extraorbital sites overall account for only approximately 25% of SC, and the head and neck region is involved in 70% of cases. Involvement of the oral cavity in SC is rare. Hereby we present a case of cutaneous SC with direct extension and involvement of the oral mucosa. Case presentation: A 25-yearold male presented with a painless ulcerated lesion on the left commissure area persisting for a duration of 3 months. The past medical history was unremarkable. Extra-oral examination showed a markedly ulcerated, exophytic, irregularly shaped mass of the left commissure extending to the labial and buccal mucosa. The lesion straddled the point of transition between mucosa and skin. The clinical differential diagnosis included squamous cell carcinoma, basal cell carcinoma, and salivary gland malignancy. On histopathological examination, a poorly differentiated malignant epithelial neoplasm with sebaceous differentiation was identified. Upon immunohistochemical testing, the neoplastic cells were strongly reactive for AE1/3, EMA, and CD15. In addition, CAM 5.2 and Ber-EP4 were also focally positive toward the periphery of the neoplastic islands. SOX10 was negative. The final diagnosis of sebaceous carcinoma was rendered. The patient was referred for oncologic treatment but lost to follow-up. Conclusion: This case illustrates a rare case of SC with extension beyond its typical cutaneous boundaries into the oral mucosa. The presentation of SC in this manner underscores the complexity of diagnosing and managing such uncommon manifestations of skin cancers. This case reinforces that SC should be included in the differential diagnosis of lesions involving both skin and oral cavity and careful evaluation of both histopathology as well as immunohistochemistry is essential for accurate diagnosis.

Poster Presentation

Division

MS/Resident

Abstract Title

The Incidence of Cemento-osseous Dysplasia and Its Contemporary Correlations

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Area of Interest

Basic Science Research

Abstract

Cemento-Osseous Dysplasia (COD) is a differential diagnosis in regards to teeth with radiolucent lesions surrounding the periapex. The University of Florida's Dental Clinic has encountered multiple cases that have been misdiagnosed and therefore mistreated. The purpose of this retrospective study is to determine the incidence of each type of COD and to quantify the number of cases that have been endodontically treated. A secondary aim is to uncover the possible correlations of patient demographics with COD. Methods: Existing imaging modalities for 77 patients diagnosed with COD were evaluated along with the radiographic interpretations and demographic information for each. Results: The findings of this study demonstrated that the most prevalent type of COD was Florid (55.8%), followed by Periapical (23.4%) and Focal (20.8%). Females (88.3%) were also more likely than males (11.7%) to be diagnosed with COD with 59% of the total number of participants being Black, followed by White (33.8%). Of the total cases, 40.3% had endodontically treated teeth and only 24.7% of those teeth had a history of sensibility tests being completed. The maxillary arch (59.2%) had a higher prevalence of root canal treated teeth in patients diagnosed with COD than the mandibular arch (40.8%) and the most common tooth type to be treated belonged to the category of maxillary anterior teeth. Conclusion: COD appears to remain prevalent in African American Females. This study revealed that sensibility testing was not routinely done prior to endodontic therapy for patients diagnosed with COD and the most common teeth to be treated endodontically were Maxillary anterior dentition.

Poster Presentation

Division

MS/Resident

Abstract Title

Comparison of Working Length Determination Between Cone-beam Computed Tomographic and Apex Locators in Posterior Teeth

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Area of Interest

Clinical Science Research

Abstract

Proper working length determination plays an important role in the success of root canal therapy. The purpose of this study was to evaluate the accuracy of CBCT (Morita Veraview X800) measurement of working lengths relative to those established clinically with an electronic apex locator (Morita Root ZX II). Eighty-six posterior teeth in which the focal view CBCT image was indicated for treatment were included in this study. Working lengths determined by the CBCT image using the measuring tool were recorded and categorized by tooth type (premolar and molar). These lengths were then compared to the working lengths determined with the Morita Root ZX II. Paired t-tests were used to determine any statistical differences among groups. There was no statistical difference when comparing CBCT working lengths to working lengths determined by the Morita Root ZX II apex locator. Tooth type (p>0.01) did not make a statistically significant difference. This study supports the use of the CBCT measuring tool as a possible adjunct or alternative to the apex locator during working length determination.

Poster Presentation

Division

MS/Resident

Abstract Title

MECHANICAL PROPERTIES OF BONDED VS. NON-BONDED CAD/CAM 3D-MILLED AND-PRINTED OVERLAYS.

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Area of Interest

Clinical Science Research

Abstract

The aim of this study was to evaluate the mechanical properties of bonded vs. non-bonded CAD/CAM 3D-milled and Printed materials. Monolithic CAD/CAM block materials, including Lava Ultimate (LU), Vita Enamic (VE), lithium disilicate ceramic, leucite-reinforced glass ceramic (LR), and composite resin for additive manufacturing (CR) were tested bonded and non-bonded. Disk-shaped test specimens with a diameter of 12 mm and a thickness of approximately 1.0 mm were tested for each group. For the bonded group, a dentine analog was used (NEMA grade G10; G10). Biaxial flexural strength (BFS) testing was performed until fracture occurred using an Instron Universal testing machine. Data was submitted to oneway ANOVA with a level of significance of 5%. In the non-bonded group, LD showed the highest BFS among all materials (312.71 ± 51.89 MPa), followed by Crown X with BFS (156.55 ± 30.88 MPa). No differences were found in the BFS between VE (107.03 ± 11.19 MPa), LU (122.72 ± 22.52 MPa), and LR (118.56 ± 22.94 MPa). In the bonded group, LD also shows the highest BFS among all materials (184.77 ± 22.27 MPa). No difference was found in BFS between LR (157.24 ± 14.9 MPa) and VE (138.39 ± 10.42 MPa). No difference In BFS was found between LU (114.96 ± 9.39 MPa) and CR (91.45 ± 8.29 MPa). The BFS between the bonded and the non-bonded groups showed significantly lower values after bonding. Lithium disilicate glass ceramics present higher BFS than leucite-reinforced glass ceramics, resin composite blocks, and composite resins for additive manufacturing. No difference was found between LR and the two resin composites. Clinicians should be aware of the limitations of leucitereinforced glass ceramics, composites CAD-CAM blocks, and 3D-printed composites mechanical properties when used as overlays.

Poster Presentation

Division

MS/Resident

Abstract Title

EVALUATION OF EARLY FAILURES ON DIRECT RESTORATIONS PLACED BY DENTAL STUDENTS

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Area of Interest

Clinical Science Research

Abstract

This study aims to evaluate and understand why amalgams, resin composites, and glass ionomers placed by dental students fail in a short period of time. Authors extracted data from an electronic health record (axiUm) with duplicate CDT codes reported on the same tooth requiring reintervention in less than five years were evaluated. Seven hundred and eighteen direct restorations were included in this study. Two examiners searched the patient's dental history, clinical forms, documentation notes, radiographs, and clinical photographs. Early failures were evaluated and associated with different variables to determine risks for failures. Statistical analysis used the One-Way ANOVA (P=0.29) and Chi-Square (p<0.00001) tests. The results with the most frequent failure were secondary caries (30%), followed by debonded restorations (26%) and fractured restorations (16%). 50% of reinterventions failed within a year. Class V resin-based composite restorations failed the most. Posterior restorations failed due to secondary caries, maxillary anterior restorations failed due to fractures of the restorations, and anterior mandibular restoration failed because of debonding. An interesting finding was that most restorations failed at reintervention for the same reason they were treated in the first place. A comprehensive oral examination for adequate diagnosis and treatment plan proves to be very important. These would provide information that would add to the understanding of the indications and contraindications for restorations, and adequate restorative techniques to minimize the risk of early failure of direct restorations.

Poster Presentation

Division

MS/Resident

Abstract Title

A CBCT Analysis of Mid Mesial Canal Morphology in Mandibular First Molars

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Area of Interest

Basic Science Research

Abstract

In recent literature, the prevalence of the mid-mesial canal in mandibular first molars has become an important topic of research, with reported incidence of the canal being present ranging from 0.2% to 44.5%. Objectives: The purpose of this study was to evaluate the occurrence of mid-mesial (MM) canals in mandibular first molars, whether they're more common in teeth with 2 distal canals, as well as the relationship of inter-canal distances with and without the presence of the mid-mesial. Methods: One hundred images were acquired with Veraview X800 Morita CBCT (pixel size= 80 µm) and evaluated according to Vertucci's classification. Two independent evaluators (oral and maxillofacial radiology and endodontic residents) evaluated the distance between the center points of the mesial canals and distal canals. Comparisons between groups were made using Student's t test and relationships between parameters were assessed by Pearson's correlation coefficient (p<.01). Results: The MM canal was present in 10 cases (10%) reviewed during this study. The average distance between the mesial-buccal (MB) canals and the mesial-lingual (ML) was greater for teeth with MM present, however it was not statistically significant. The distance from mesial canals to distal canals was also greater in the MM group, but not statistically significant. Of the teeth with a MM canal present, the majority (90%) were Vertucci class 2 or 3, joining either the MB or ML canal before the apical exit. Conclusion: A greater inter-canal distance in mandibular molars may suggest the presence of a third mesial canal (mid-mesial).

Poster Presentation

Division

MS/Resident

Abstract Title

Odontogenic Myxoma in a Patient with MEN2A Syndrome

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Area of Interest

Clinical Science Research

Abstract

Introduction: Odontogenic myxoma is considered to originate from the odontogenic ectomesenchyme. The tumor can involve any age group, but is predominantly seen in young adults. Here we present a patient with odontogenic myxoma with a history of multiple endocrine neoplasia type 2A (MEN2A). To the best of our knowledge, odontogenic myxoma has not been previously reported in a patient with a MEN2A history.

Case presentation: A 14-year-old female with a known history of MEN2A, presented with a painless bony expansion and radiolucency with fine residual bone trabeculae arranged at right angles to one another between teeth #4 and #5. Upon biopsy a diagnosis of odontogenic myxoma was rendered. MEN2A is an autosomal dominant syndrome characterized by clinically aggressive medullary thyroid carcinoma (MTC), pheochromocytoma, parathyroid hyperplasia, or adenoma. Therefore, as the standard of care for the MTC includes prophylactic total thyroidectomy, it was undertaken in this patient at an early age. For the odontogenic myxoma, the patient was treated with enucleation and curettage. At 2 years post-resection follow-up, the patient was disease-free.

Conclusion: It is unclear if the odontogenic myxoma in this patient was associated with previously diagnosed MEN2A syndrome or incidental, but the possibility of associated mesenchymal tissue tumors should be considered in patients with history of syndromic conditions. Although odontogenic myxoma is a benign lesion, because of its high recurrence rate and locally aggressive clinical behavior, periodic follow-up is necessary for at least 5 years post-treatment. Despite the high recurrence rate of 25% as reported in the literature, the prognosis largely remains good.

Poster Presentation

Division

MS/Resident

Abstract Title

Mucinous Adenocarcinoma of The Lateral Tongue with Benign Clinical Appearance: A Diagnostic Challenge

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Area of Interest

Clinical Science Research

Abstract

Introduction: Mucinous adenocarcinoma (MAC) of the tongue is an exceedingly rare malignancy, with only a limited number of cases reported worldwide. It represents a distinct and heterogeneous subtype of adenocarcinoma characterized by mucin production by the tumor cells. We present a unique case of mucinous adenocarcinoma of the tongue, which exhibited a benign clinical appearance.

Case presentation: A 76-year-old male presented with a painless white irregular hyperkeratotic lesion on the left lateral dorsal aspect of the tongue, which was diagnosed as severe hyperparakeratosis and leukoedema. The lesion recurred in the same location 1.5 years later with a clinical impression of fibroma. A subsequent second recurrence was noted and diagnosed as high-grade MAC exhibiting both perineural and intravascular invasion. Immunohistochemical staining demonstrated a strong positive reaction to CK7, AE1/3, and mucicarmine stains and negative staining with CK20. Melanoma markers (HMB45, S100, SOX 10, and Melan A) were negative. Stains to rule out possible lung metastases (TTF-1, P-63, Napsin-A), and alternate salivary gland tumors (GATA-3, and p40) were also negative. The final diagnosis of high-grade MAC was made according to AFIP criteria. The patient was referred for oncology assessment and treatment but lost to follow-up.

Conclusion: MAC of salivary gland origin infrequently arises within the oral cavity. Salivary tumors on the lateral tongue are also extremely rare. This case represented a diagnostic challenge due to the initially benign clinical appearance and histological overlap with other more common salivary malignancies such as mucoepidermoid carcinoma, other adenocarcinomas, and metastatic lesions. Utilizing objective histopathologic features to diagnose MAC is pivotal to facilitate timely and appropriate management.

Poster Presentation

Division

MS/Resident

Abstract Title

Early treatment practices and perceptions among orthodontists in the United States: A survey-based study

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Area of Interest

Clinical Science Research

Abstract

Objective: To explore early treatment practices and perceptions among members of the American Association of Orthodontists (AAO). Materials and methods: An electronic questionnaire was pre-piloted and circulated via email to members of the AAO. The survey included questions related to demographics, clinical experience, timing, treatment approaches to early treatment, and clinicians' perceptions. Descriptive statistics were performed. Results: A total of 226 responses were obtained. Treatment was commonly timed using dental age (61%) and pubertal signs (61.8%). Several problems were treated in the mid-mixed dentition phase (8 - <10 years) including posterior crossbite (51.3%) increased overjet (41.7%), crowding (39.5%), space loss (41.23%), and anterior open bite (37.3%). Commonly reported treatment approaches included the use of sectional fixed for treatment of anterior crossbite (81.6%), rapid palatal expander for treatment of posterior crossbite (63.6%), and anterior bite plane for deep overbite correction (62.7%). Additionally, Herbst appliance (42.1%) and facemask (89%) were commonly prescribed for early correction of Class II and III, respectively. The majority of respondents were proponents of two-phase orthodontic treatment (60.1%), and the highest perceived success of early treatment was rated for anterior (mean ± standard deviation (SD): 6.7 ± 2.3) and posterior crossbite (mean ± SD: 6.5 ± 2.2) correction. Lingual and palatal arches were commonly used to preserve arch length. Conclusions: Early orthodontic treatment is widely advocated for various malocclusions. Future clinical trials assessing the benefits, potential harms, and stability of outcomes would be valuable for further understanding and refinement of treatment approaches.

Poster Presentation

Division

MS/Resident

Abstract Title

Familial Gigantiform Cemantoma in a Young Haitian Female: Case Report of a Rare Entity

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Area of Interest

Clinical Science Research

Abstract

Introduction: Familial Gigantiform Cementoma (FGC) is a rare fibro-osseous lesion affecting the gnathic bones, with fewer than 40 cases reported. FGC typically exhibits rapid and aggressive growth often resulting in grotesque facial distortion. FGC usually begins in childhood and is often familial with an autosomal dominant inheritance pattern. Although rare sporadic cases have been reported. FGC may involve more than one bone in the head and neck. Recent studies have identified molecular mutations in FGC similar to those reported in gnathodiasphyseal dysplasia.

Case presentation: A 15-year-old Haitian female presented with massive swelling over the left side of face, accompanied with severe headache and intermittent burning of her eyes. She had history of multiple facial growths since 8 years of age which had been treated surgically in the past. No family history, surgical or medical history were available. Clinical examination revealed a large mass of left mandible measuring approximately 9 x 9.5x 7.1 cm that was radiographically well-circumscribed but expansile and exhibited diffuse mineralization of facial bones, skull base, and calvarium. Microscopic examination revealed trabeculae of variably mineralized cemento-osseous calcification with osteoid formation distributed within a hypercellular fibrovascular stroma. The calcifications exhibited osteoblastic rimming and occasional multinucleated osteoclastic cells. A diagnosis of benign fibro-osseous lesion compatible with FGC was rendered. The patient was lost to follow up.

Conclusion: FGC poses a distinct diagnostic challenge owing to clinical and histologic overlap with other benign fibro-osseous lesions. Clinical and radiographic correlation is important. Additionally, treatment options are limited since complete surgical resection which is definitively curative, is not always possible and often impractical. Conservative management typically results in recurrence with several patients facing significant disfigurement and compromised quality of life. These factors emphasize the importance of accurate and early diagnosis. Management requires a multi-displinary approach.

Poster Presentation

Division

MS/Resident

Abstract Title

Selective Bracket Bonding for Torque correction

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Area of Interest

Clinical Science Research

Abstract

The spatial position of the roots is absent from the digital setups in clear aligner therapy. The emphasis in the simulated treatment plan is on the crown position rather than the root position. It has been illustrated in the literature, that the 3D clear aligners' digital setups might push the teeth outside the bony envelope. 1 This pearl illustrates the management of a root that has been pushed out of the bone boundary following clear aligner therapy. The goal in such a case would be to reposition the affected root by torque control into the alveolar bone. This demands the use of fixed appliances and altering the choice of brackets based on the used brackets' prescription. However, full torque expression is never achieved until a full-dimensional Stainless-Steel wire is inserted into the bracket. In addition to bracket variation, torque auxiliaries, and third-order bends can be added for better torque expression.

Technique

In this example, the upper left first premolar root was pushed buccally outside the bony envelope after clear aligners' therapy. A CBCT radiograph was taken to confirm the diagnosis (Figure 1- A and B). The decision was made to place a sectional fixed appliance to correct the position of the root. An MBT 022" slot maxillary incisor bracket was used on the upper left first premolar tooth to maximize the lingual root torque (+17°). The adjacent teeth (upper left canine and 2nd premolar teeth) were also bracketed. A sequence of 0.018×0.018-inch Bioforce, 0.019×0.025-inch nickel-titanium, and 0.019×0.025-inch SS wires were used. The insertion of the rigid thick rectangular archwire "0.019×0.025-inch SS" in the upper left first premolar was pushed lingually (Figure 2 - A and B). The torque was corrected within approximately 5 months.

Torque control was therefore achieved using a full dimensional SS archwire, inserted into a central incisor's bracket.

Poster Presentation

Division

MS/Resident

Abstract Title

Educational Benefits of Continuing Education in Shade Matching

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Area of Interest

Clinical Science Research

Abstract

Educational Benefits of Continuing Education in Shade Matching

Objectives, The goal of the study was to determine the current status of the knowledge of color in dental education at the University of Florida and the influence of additional training on their education in shade matching in dentistry.

Methods, A cross-sectional web-based survey, containing 10 multiple choice questions was created. Upon receiving the Institutional Review Board approval (IRB202300154), dental professionals from the University of Florida College of Dentistry (N=330) were administered the survey. Forty participants (28 female, 12 male) were selected to participate in this study based on representativeness, and randomly divided into two groups (n = 20), experimental and control. Participants in the experimental group were invited to undergo additional training in shade matching in dentistry, and then retake the web-based survey. Participants in the control group were requested to retake the web-based survey without the additional training in between. Additional training consisted of taking online didactic training offered by the Society for Color and Appearance in Dentistry, followed by an in person hands-on workshop. Statistical analysis of differences between knowledge from participants in experimental and control groups was performed using ANOVA test (α =0.05).

Results, A total of 105 responses were received from the web-based survey administered (response rate 32%); the average score obtained was 40%. The participants selected to participate in the study had to have an average score similar to the average dental professionals at UFCD (p=0.52). Following additional training in shade matching, scores of participants in the experimental group were significantly higher than those in the control group (p=0.01).

Conclusions, The results of this study highlight the importance of continuous education and specialized training in improving the competencies of dental professionals, particularly in areas that directly impact patient satisfaction and clinical outcomes.

Poster Presentation

Division

MS/Resident

Abstract Title

Quantitative Analysis of Biofilm Removal Following Irrigation with EndoActivator and GentleWave Systems: Microbiological Study

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Area of Interest

Basic Science Research

Abstract

Introduction: Mesial roots and isthmuses of mandibular molars are difficult areas to obtain adequate disinfection of root canal walls, and consequently microorganisms can survive treatment. Objectives: The present study compared, through real-time polymerase chain reaction (qPCR), the effectiveness of EndoActivator (EA) (Dentsply Sirona, York, PA, USA) and GentleWave System (GWS) (Sonendo Inc, Laguna Hills, CA) in removing Enterococcus faecalis (E. faecalis) from the mesial canals and isthmuses of mandibular molars.

Methods: Thirty extracted human lower molars were inoculated with E. faecalis OG1RF for 14 days, and then an initial bacterial sample was collected with paper points from mesiobuccal and mesiolingual canals and isthmuses. The specimens were randomly divided into four groups (n = 10 teeth; 20 canals each), according to irrigation systems: EndoActivator and GentleWave. The remaining 10 teeth were divided between positive control, inoculated teeth without instrumentation or irrigation, and negative controls, teeth without inoculation. After instrumentation, the final sample was taken using paper points and DNA was isolated. Primers specific for E. faecalis were used for qPCR. The bacterial reduction between pre- and post-instrumentation was calculated. One-way analysis of variance (ANOVA) with Tukey post hoc tests were for statistical analysis with significance of (p < 0.05).

Results: Both irrigation systems were able to reduce the load of E. faecalis from the prepared root canals. Conclusion: There was no significant difference between the effectiveness of the EndoActivator and the GentleWave in removing E.faecalis from the mesial canals and isthmuses of mandibular molars.

Poster Presentation

Division

PhD/Post-doc

Abstract Title

Cryptococcus neoformans shows increased pathogenesis in mice after interactions with Staphylococcus aureus

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Area of Interest

Basic Science Research

Abstract

Background. Cryptococcus neoformans (Cn) is an opportunistic, neurotropic, and encapsulated fungus that causes more than 150,000 worldwide cases of cryptococcal meningitis in people living with HIV/AIDS each year. Methicillin-resistant Staphylococcus aureus (Sa) colonization and infections have increased in the last years and an 18-fold higher rate are reported in people living with HIV/AIDS. Polymicrobial interactions trigger synergistic or antagonistic effects in the virulence of microbes. The interaction between Cn and Sa is possible since both can colonize and infect the skin, lungs, and brain. Furthermore, it is possible that environmental Cn-Sa interaction can generate virulent strains transmissible to humans. We hypothesized increased Cn pathogenesis in mice after interaction with Sa.

Methods. Cn strain H99 and Sa strain NR45992 were used in vitro and in a pulmonary co-infection mouse model. Growth curves, Cn capsule measurements, proteomic analysis of the secretome, and scanning electron microscopy were performed during Cn-Sa interactions in vitro. Balb/c mice were intratracheally infected with 105 CFU of Cn, Sa, or Cn and Sa and survivability studies were performed. To assess disease progression, fungal load and capsule size determinations, histopathology, and cytokine measurements were evaluated in lung and brain tissue.

Results. Cn capsule size was significantly reduced in co-culture with Sa conditions compared to single culture after 6-, 24-, and 48-hours. Unique proteins involved in Cn capsule synthesis were identified in the secretome of 24-h co-cultures. In contrast, superantigens, enterotoxins, and antibiotic resistance proteins were produced by Sa after interactions with Cn. Co-infected mice showed high bacterial and fungal burden in brain tissue at 3- and 7-days post-infection. However, only a significantly high fungal burden was found in the lungs. Surprisingly, Cn capsule size was significantly enlarged in co-infected mice at 3- and 7-days post-infection.



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PHD/POSTDOCTORAL

ABSTRACT PRESENTATIONS







Conclusion. Our findings suggest that polymicrobial interactions influence the regulation and production of virulence factors by individual microorganisms, important elements needed for their survival and successful colonization of the human host.

Poster Presentation

Division

PhD/Post-doc

Abstract Title

Sweetening the Deal: Glycosylation of Proteins by the Pgf Machinery is Important for Streptococcus mutans Pathophysiology

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Abstract

Objectives: Identify physiological aspects of Streptococcus mutans that require adequate glycosylation by the Pgf glycosylation machinery, encoded in the core genome as a four-gene operon (PgfS-PgfM1-PgfE-PgfM2), and elucidate its mechanism. Determine interaction partners of each Pgf protein to design an interactome network that links to known biochemical pathways of S. mutans.

Methods: Phenotypical characterization of mutants lacking each pgf gene or the entire pgf operon under various stresses and pathogenicity-related circumstances such as cell envelope homeostasis, biofilm formation, sensitivity to human saliva, and oral colonization ability in a rat model. Identification of interaction partners of each Pgf protein with a bacterial two-hybrid approach. Assays had plasmids encoding each Pgf protein as bait and predicted/hypothesized targets (direct approach) or a DNA library (indirect approach) as prey.

Results: The Pgf machinery ensures proper biofilm formation, impacting its thickness, volume, and distribution. Cell envelope-related phenotypes such as sensitivity to detergent SDS and to the rhamnose-glucose polysaccharide (RGP) synthesis inhibitor tunicamycin were also observed. In silico analyses and the sensitivity of mutants to tunicamycin suggest functional redundancy of the Pgf system and the synthesis of the serotype-determiner RGP layer. Mutants were more sensitive to saliva-mediated killing and had severely impaired oral cavity colonization ability in a rat model. PgfE, a GlcNAc-GalNAc 4-epimerase, forms a homodimer. Glycosyltransferase PgfS binds to known substrates Cnm and WapA, and also with the major biofilm-forming exoenzyme GtfB. It also interacts with the threonine-kinase PknB, suggesting a novel crosstalk between protein glycosylation and protein phosphorylation in bacteria. Transmembrane proteins PgfM1 and PgfM2 also interact with Cnm and WapA, with PgfM1 additionally interacting with biofilm exoenzymes GtfB and GtfC and the latter with GtfB. Preliminary indirect two-hybrid assays using PgfS as bait suggest interactions with FtsK, PlsC, PtsK, AdcA, and FruB. Conclusions: The Pgf machinery is linked to essential physiological pathways, impacting bacterial physiology and virulence. This makes it a promising target for drug development against the cariogenic bacterium Streptococcus mutans.

Poster Presentation

Division

PhD/Post-doc

Abstract Title

Novel Nitric Oxide-Releasing Pluronic F-127 Organogel Inhibits Progression of Melanoma

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Area of Interest

Basic Science Research

Abstract

Melanoma is a malignant neoplasm of melanocyte cell origin located on the skin. While rare, it can also occur in the oral cavity, which unfortunately progresses rapidly and proves to be aggressive. Excision is the only curative treatment whereby local recurrence and metastasis are significant risks. Thus, there are still clinical needs for novel interventions, where nitric oxide (NO), an endogenous gasotransmitter, represents an attractive approach. NO can exert tumorigenic or tumoricidal effects at low (nM) and high (µM) concentrations, respectively and thus highlight the need for controlled delivery. Our group has developed a novel amine-modified cyclodextrin NO donor (CD-EOH7/NO) and optimized a Pluronic F-127 organogel formulation for its topical application. Pluronic F-127 organogel was selected as it is a liquid at cold temperatures and solidifies into a cream-like consistency when applied to the skin. Objectives: The objective of this study was to examine the in vivo efficacy of CD-EOH7/NO in controlling tumor growth and delineation of associated mechanisms. Methods: Syngeneic B16-F10 melanoma tumor cells were injected subcutaneously into 8-week-old C57BL/6 female mice which were treated daily with topical or subcutaneous applications of CD-EOH7/NO. Results: Up to date 7, mice receiving topical application of CD-EOH7/NO showed reduced tumor size compared to all controls, suggesting that NO slows the progression of melanoma growth. The largest difference was observed at day 7, where mice receiving topical CD-EOH7/NO had an average tumor size of 10.3 mm2, and mice receiving no treatment had an average tumor size of 20.1 mm2. In addition, mice receiving subcutaneous administration of CD-EOH7/NO presented with no or minimal (<3mm2) tumor growth up to 10-days post tumor cell injection. Conclusions: These findings suggest that topical delivery of NO has promise as a novel therapeutic for malignant melanoma lesions, while subcutaneous administration of NO has potential for controlling progression of melanoma following excision.

Poster Presentation

Division

PhD/Post-doc

Abstract Title

Altered OMV Binding and TLR Innate Immune Sensing of Porphyromonas Gingivalis via Microbial Sphingolipid Synthesis

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Authors

Z. G. Eure*, F. Rocha, J. Solbiati, F. C. Gibson III

Area of Interest

Basic Science Research

Abstract

Objective: Periodontal disease (PD) is a chronic inflammatory disease initiated by a dysbiotic subgingival microflora. Porphyromonas gingivalis (Pg) is routinely associated with PD and is capable of manipulation of host immunity. Microbial sphingolipids (SLs) are detected in varying proportions in periodontal health and disease. Our group reported that Pg modulates the host inflammatory response by transferring its SLs to host cells and this occurs in a contact independent manner via outer membrane vesicles (OMVs). Pg innate immune sensing is associated with OMV binding to host cells. Pg and its OMVs and known to be sensed predominantly by Toll-like receptor 2 (TLR2), which requires Myeloid differentiation factor 88 (MyD88) for signaling. The objective of this study is to define the role of SLs in Pg elicited inflammation and to investigate how Pg sphingolipids limits host inflammation may be linked to targeting of MyD88. Methods: Pg W83 and W83 SPT (SL deficient mutant) was grown anaerobically in broth culture, washed, and suspended in RPMI 1640 cell culture medium without antibiotics. Pg W83 WT and W83 SPT OMVs were isolated from bacteria grown in RPMI 1640 medium aerobically and purified via density-gradient ultracentrifugation. THP-1 macrophages were challenged with DiOlabelled OMVs at 500, 1000, 2000 OMVs/cell and percentage of OMV+ cells were quantified via fluorescence microscopy. Reporter cells (THP-1 Dual and HEK-Blue) (Invivogen) were challenged with Pg W83 or SPT (1000 OMVs/cell). Secreted embryonic alkaline phosphatase (SEAP) was detected per manufacturer's instructions. Cell lysates from untreated, Pam3CSK4 treated, or Pg W83 (WT or ∆SPT) treated THP-1 macrophages were collected for western blot analysis. MyD88 protein expression was normalized to -actin. Results: Pg OMV SLs reduced OMV binding to THP-1 macrophages in a dose-independent manner. TLR2 is predominantly involved in Pg W83 OMV sensing and SLs limit OMV TLR-signaling potency. THP-1 cells rely on MYD88, in contrast to TRIF, to activate NF-kB and SLs limit MyD88-dependent NF-kB-signaling effectiveness. Preliminary data suggest SLcontaining Pg W83 promotes reduced MyD88 protein levels. Conclusion: While future studies are ongoing, our data have highlighted sphingolipids to be involved in inhibiting OMV binding to macrophages and limiting innate immune sensing of Pg OMVs. In addition, our data have suggested a potential Pg mechanism to modulate inflammation by targeting MYD88.

Poster Presentation

Division

PhD/Post-doc

Abstract Title

Comparative Analysis of Sleep Quality Assessment: A Study on the Pittsburgh Sleep Quality Index (PSQI) and the OURA© Ring in Middle to Older Aged Adults with Chronic Pain.

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Area of Interest

Clinical Science Research

Abstract

Sleep quality is a critical aspect of health and well-being, particularly among middle to older-aged individuals experiencing chronic pain. While self-reported measures like the Pittsburgh Sleep Quality Index (PSQI) are commonly used, the advent of wearable technology like the OURA© Ring offers objective sleep data collection. This study aims to compare these two contrasting yet complementary methods of evaluating sleep quality. We used baseline data from a pilot clinical trial that employed comprehensive objective and self-reported sleep assessments in persons with chronic musculoskeletal pain (i.e., pain greater than 4 out 10 during the past 3 months on most days) and self-reported sleep disturbance (PSQI score > 5, n=24). We estimated measures and components equivalent to the PSQI questionnaire (e.g., the 30-day average of sleep and bed hours, sleep quality, latency, duration, efficiency, and disturbances) from objective OURA© ring data to evaluate whether self-reports in the PSQI questionnaire reflecting objective sleep states and patterns. Pearson's correlation coefficient was used to assess the correlation between the PSQI measures and scores and their OURA© Ring equivalents. There were statistically significant correlations between several pairs of sleep measures from the PSQI and their equivalents from the OURA© Ring. Notably, there was a moderate positive correlation for Sleep Timing (r=0.503, p=0.012, p'=0.049), a strong positive correlation for Total Hours of Sleep (r=0.705, p=0.000, p'=0.00048), a very strong positive correlation for Time to Wake Up - Time to Go to Bed (r=0.833, p=0.000, p'=1.70E-06), and a strong positive correlation for Sleep Duration (r=0.646, p=0.001, p'=0.0033). Corrected by Holm-Bonferroni method. Our results show significant associations between self-reported subjective and objective measures of sleep quality in middle to older-aged adults with chronic pain and sleep disturbances. Our research underscores the importance of integrating subjective and objective measures in sleep research. It contributes to a comprehensive understanding of sleep quality assessment and its implications on health and well-being among aging populations experiencing chronic pain. Psychological, cognitive, and neurobiological factors may influence the subjective self-reported PSQI answers. Our pilot data supports this, and we aim to identify these discrepancy causes and their role in objective-subjective relationships.

Division

PhD/Post-doc

Abstract Title

Contrasting the genome-wide transcriptional roles of the four viral interferon regulatory factors of KSHV in oral epithelial cells

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Area of Interest

Basic Science Research

Abstract

The viral interferon regulatory factors (vIRFs) of KSHV were shown to dysregulate cell signaling pathways to promote viral oncogenesis, and immune evasion strategies of KSHV to facilitate infection. However, it remains unknown to what extent each vIRF plays different roles in gene regulation. To address this, we performed a comparative analysis of the protein structure and gene regulation potential of all four vIRFs. Our structure prediction analysis revealed that despite their low amino acid sequence similarity, vIRFs display a high structural homology in their DNA-binding domain (DBD) and IRF association domain (IAD). Despite this structural similarity, we demonstrate that each vIRF regulates a distinct set of KSHV gene promoters and human genes in epithelial cells. We also determined that the DBD of vIRF1 is essential for regulating the expression of its target genes. We propose that the structurally similar vIRFs evolved to possess specialized transcriptional functions regulating different genes.

Additional Information

I regret to inform you that I will not be able to participate in the poster presentation due to my commitment to attend a colleague's PhD final defense at the Toth Lab.

Poster Presentation

Division

PhD/Post-doc

Abstract Title

MicroRNA Expression Profiling in Fusobacterium nucleatum-infected Periodontitis and Machine Learning

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Area of Interest

Basic Science Research

Abstract

Objectives: Fusobacterium nucleatum is a major intermediate colonizer comprising the subgingival pathogenic polymicrobial community and is implicated in systemic diseases. F. nucleatum can coaggregate with late colonizing periodontal bacteria (Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia). The objective of this study is to determine global microRNAs (miRNA, miR) expression kinetics (8- and 16-weeks) in F. nucleatum ATCC 49256-infected male and female C57BL/6J mice. Methods: Both male and female mice were divided into four groups (n=10) (Fn-infected-8 weeks; sham-infected-8 weeks; Fn-infected-16 weeks; sham-infected-16 weeks). In each group, bacterial infection was performed (infection cycle four days per week), mice euthanized, and tissues harvested. We evaluated differentially expressed (DE) miRNAs in mandibles (n=10) using high throughput NanoString nCounter® miRNA expression panels. Bacterial colonization/infection and alveolar bone resorption (ABR) were measured. Results: All infected mice (n=10) showed bacterial colonization in the gingival surface and significant increase in ABR (p<0.01). miRNA profiling showed 7 upregulated miRNAs (e.g., miR-361, miR-26a, miR-99b) and 2 downregulated miRNAs (e.g., miR-362-3p, miR-720) during 8-weeks of infection, whereas 7 upregulated miRNAs (e.g., miR-let-7a, miR-127, miR-361, miR-99b) and 13 downregulated miRNAs (e.g., miR-323-3p, miR-488) during 16-weeks infection. miR-361 and miR-99b were upregulated in both 8- and 16-weeks infected F. nucleatum mice mandibles and all DE miRNAs were unique to the specific time point. KEGG predicted pathway analysis of 5 DE miRNAs (let-7a-5p, miR-361-5p, miR-345-5p, let-7f-5p, miR-218-5p) were associated in bacterial invasion of epithelial cells. Additionally, the machine learning algorithms XGBoost (eXtreme Gradient boost) and random forest were used to predict the association between the number of miRNA copies whether the mouse was infected. Conclusions: miRNA expression kinetics is unique in 8- and 16-weeks infection and is time-dependent during periodontitis. Differential miRNA expression pattern is observed between F. nucleatum-infected male and female mice. F. nucleatum monobacterial infection-induced miRNAs are distinctive from polybacterial infection-induced miRNAs.

Poster Presentation

Division

PhD/Post-doc

Abstract Title

Partners in Grime: Exploring the synergism between Candida albicans and Streptococcus mutans in oxidative stress tolerance

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Area of Interest

Basic Science Research

Abstract

Objective: Co-infection with Streptococcus mutans and Candida albicans is associated with dental caries. When these two microorganisms are co-cultivated, they exhibit enhanced environmental stress tolerance, including heightened oxidative stress tolerance for S. mutans. Candida albicans is highly tolerant to oxidative stress as it expresses catalase and three extracellular superoxide dismutases (SOD), enzymes that detoxify H2O2 and superoxide. However, S. mutans encodes only a single SOD. The purpose of this study is to investigate the role of C. albicans' catalase and SODs in the protection of S. mutans-C. albicans biofilms against oxidative stress.

Methods: C. albicans SC5314 (CaWT), its catalase mutant (Ca Δ cat), its triple extracellular SOD muant (Ca $\Delta\Delta\Delta$ SOD), and S. mutans UA159 (SmWT), were individually or co-cultivated in BHI + 1% sucrose (BHIS) on hydroxyapatite disks and subjected to time-kill assay using 0.25% H2O2 + 1mM FeSO4 after 48hrs. CaWT, Ca Δ cat, Ca $\Delta\Delta\Delta$ SOD, and SmWT, were individually or co-cultivated in cell-free saliva + 1% sucrose (BHIS) on hydroxyapatite disks and subjected to time-kill assay using 0.1% H2O2 after 48hrs. For biofilm dispersion assays, CaWT, Ca Δ cat, Ca $\Delta\Delta\Delta$ SOD, and SmWT, were individually and co-cultivated in BHIS on saliva-coated plates. After 8hs, biofilms were treated with 0.005% H2O2 or a commensal, Streptococcus A12, was introduced. Then, 10hrs later, biofilm staining for biomass quantification was completed.

Results: Survival rate of SmWT in a dual-species BHIS biofilm in the presence of CaWT was greater than the survival rate when co-cultivated with Ca Δ cat, Ca $\Delta\Delta\Delta$ SOD or when grown as a mono-species biofilm (p<0.01). Ca Δ cat and Ca $\Delta\Delta\Delta$ SOD were hypersensitive to oxidative challenge in comparison to CaWT (p<0.05). S. mutans survival in saliva was also enhanced by the presence of CaWT in dual-species biofilms; however, its survival was not enhanced by Ca Δ cat or Ca $\Delta\Delta\Delta$ SOD (p>0.01). Biofilm biomass reduction was observed by adding sublethal 0.005% H2O2 or Streptococcus A12 to SmWT as mono-species or dual-species with Ca Δ cat and Ca $\Delta\Delta\Delta$ SOD, but not with CaWT (p<0.001).

Conclusion: Catalase and extracellular SODs produced by C. albicans protect S. mutans against oxidative stress in both BHIS and saliva formed biofilms and peroxigenic commensal bacteria.

Additional Information

DMD/PhD student

Poster Presentation

Division

PhD/Post-doc

Abstract Title

Movement-Evoked Pain is Associated with Baseline and Longitudinal Cognitive Function in Individuals with Knee Osteoarthritis

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Area of Interest

Clinical Science Research

Abstract

Objectives: Emerging evidence suggests that movement-evoked pain (MEP) exhibits unique cross-sectional associations with physical function beyond traditional pain measures (e.g., pain severity, distribution) in chronic pain populations, however, the longitudinal association between MEP and physical function has yet to be fully characterized. Further, the association between MEP and cognitive function is unknown, despite the well-established relationship between chronic pain and cognitive function. Therefore, the purpose of this study was to determine if MEP is associated cross-sectionally and longitudinally with physical and cognitive function in individuals with knee osteoarthritis (OA).

Methods: This study was a secondary analysis of participants with symptomatic knee OA (N = 74, Mean Age = 58 years, 51 female) enrolled in a multisite longitudinal study at the University of Florida and University of Alabama, Birmingham. Lower extremity physical function was assessed using the Short Physical Performance Battery (SPPB), which assesses three domains of physical function: balance, walking, and chair stands. Cognitive function was assessed using the Montreal Cognitive Assessment (MoCA), which provides a measure of global cognitive function. MEP was assessed by having participants rate their pain during each SPPB task using a visual analog scale that ranged from 0 (no pain) to 100 (worst pain imaginable). Pain ratings were summed across tasks to create a total MEP score. Measures were assessed at baseline and at a two-year follow up visit. Associations between baseline MEP and baseline and two-year change scores for the SPPB and MoCA were evaluated with multiple linear regression models adjusting for study site, age, sex, race, highest education level, and body mass index. Results: MEP scores were associated with baseline ($\beta = -0.387$, p < 0.001) and two-year change ($\beta = 0.263$, p = 0.024) MoCA scores. The association between MEP scores and baseline MoCA scores remained significant after controlling for covariates (β = -0.325, p = 0.004). Baseline MEP was not associated with any SPPB score. Conclusions: MEP is associated with both baseline and longitudinal measures of cognitive, but not physical, function in this sample of individuals with knee OA. These results suggest that interventions focused on reducing MEP may be effective in reducing cognitive impairment in this population.

Poster Presentation

Division

PhD/Post-doc

Abstract Title

The effect of protective liners on pulp temperature rise during light-curing procedures.

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Area of Interest

Basic Science Research

Abstract

Objectives: To evaluate the effect of protective liners and filling dental materials on pulp temperature rising during light-curing procedures.

Material and Methods: Deep-large Class I preparations (5 mm x 4 mm and 4 mm depth) were made in third molars leaving \pm 0.5 mm of dentin thickness at the pulpal floor. Teeth were placed in a "customized oral cavity chamber circulating bath" in which humidity and temperature were standardized at 34oC. Pulp chamber temperature variations during light-activation were recorded using an infrared camera with thermal sensitivity of <0.10oC and 150 mK. The materials evaluated were: 1- Activa (Pulpdent/ 2 layers/ 2 mm thick); 2- TheraCal (Bisco) + Fuji II LC (GC); 3 Dycal (Dentsply) + Fuji II LC (GC); 4- Fuji II LC (GC/ 2 layers/ 2 mm thick). Light-activation was performed with Valo (Ultradent) according to manufacturer's instructions. A software (FLIR Tools) transferred the video database to a data sheet. Power analysis was conducted to determine sample size and power of 0.8 at α =0.05 (β =0.2). Data normality and homoscedasticity were done with Shapiro-Wilk and Levene's test, respectively. Statistical analyses were performed with ANOVA and Tukey for multiple comparison.

Results: The pulp chamber temperature increased above the 5.5°C threshold difference for all liners and fillings tested. None of the materials could provide thermal insulation for light-curing procedures. The more light-activated steps, the longer the pulp remained at a temperature above the 5.5°C threshold.

Conclusions: Light-activation of protective liners and fillings can harm the pulp due to the summation of heat development. Dycal was not capable of providing proper thermal insulation.

Abstract Title

Inhibition of RhoA Prevents Cryptococcus neoformans Capsule Glucuronoxylomannan-stimulated Brain Endothelial Barrier Disruption

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Area of Interest

Basic Science Research

Abstract

Cryptococcus neoformans (Cn) is an opportunistic fungus capable of causing severe central nervous system (CNS) disease in immunocompromised individuals. Invasion of the brain parenchyma requires fungal traversal of the selectively impermeable blood-brain barrier (BBB). Cn harbors diverse mechanisms by which it interacts with and crosses the BBB endothelium layer including transcytosis, paracellular transit, and/or the Trojan horse. In this study, we describe that Cn alters the brain endothelium by activating small GTPase RhoA, which facilitates reorganization of the actin cytoskeleton and tight junction modulation to regulate endothelial barrier permeability. We confirm the role of the main fungal capsule polysaccharide glucuronoxylomannan in initiating these alterations, which are inhibited by targeting RhoA. We reveal a therapeutic benefit of RhoA inhibition by CCG-1423 in vivo. By restoring the integrity of the BBB endothelium through RhoA inhibition, prolonged survival and reduced fungal burden is observed in a murine model of disseminated cryptococcosis, supporting the therapeutic potential of this compound in the context of cryptococcal infection. This study examines the complex virulence of Cn in establishing CNS disease and describes cellular components of the brain endothelium that may serve as molecular targets for future antifungal therapies to alleviate the burden of life-threatening cryptococcal CNS infection.

Poster Presentation

Division

PhD/Post-doc

Abstract Title

Investigation of Viable But Non-Culturable State of Porphyromonas Gingivalis and Host Cell Invasion

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Authors

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Area of Interest

Basic Science Research

Abstract

Investigation of Viable But Non-Culturable State of Porphyromonas Gingivalis and Host Cell Invasion The bacterium Porphyromonas gingivalis (Pg) is a Gram-negative, black-pigmented colony forming anaerobic pathogen known for its ability to form biofilms. It is a primary culprit in periodontal disease and has been implicated in various systemic conditions such as atherosclerosis, Alzheimer's disease, and certain types of cancer, including pancreatic and oral cancers. Pg employs various mechanisms to evade stress. In this study, we explore a phenomenon known as the Viable but Non-Culturable (VBNC) state, which has been described in aerobic bacteria but not yet established in anaerobic bacteria like Pg. VBNC denotes a state where bacteria remain viable, albeit with reduced metabolic activity, but are unable to be cultured conventionally in forming colonies on plates. To induce the VBNC state in Pg, we subjected the bacteria to oxidative stress with a known stressor, and countered its effects later for the bacteria resuscitation. Our Live/Dead assays, conducted through Live/Dead kits, confocal microscopy fluorescence imaging, and Flow Cytometry live/dead bacterial counts, confirmed the presence of significant numbers of above 50% living Pg cells, both with and without stressors and subsequent resuscitation.

However, when cultured on routine Blood Agar Plates (BAPs), the stressed Pg failed to form colonies after 7 days, indicating entry into the VBNC state. Conversely, the non-stressed and resuscitated Pg displayed significant colony formation even at high dilution factors, suggesting their continued culturability. We further investigated the invasiveness of Pg in the VBNC state using three different cell lines: HCAEC, HMVAC, and Glioblastoma. Pg in the VBNC state demonstrated the ability to infect all three cell lines, with a considerable portion of the bacteria successfully invading and persisting for more than 6 hours within the host cells.

Our objective is to utilize mRNA analysis to elucidate the pathways affected during the VBNC state of this opportunistic oral pathogen. This information will be instrumental in guiding scientists toward developing therapies for the treatment of periodontitis caused by Pg.

Poster Presentation

Division

PhD/Post-doc

Abstract Title

Dissecting the role of FOXK transcription factors during KSHV reactivation in Primary Effusion Lymphoma

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M. Palmer*, N. Atyeo, A.Leo, B. Papp

Area of Interest

Basic Science Research

Abstract

Objectives: Kaposi's sarcoma-associated herpesvirus (KSHV) is an oncogenic herpesvirus with a biphasic lifecycle consisting of a latent and lytic stage. KSHV as a true oral herpesvirus, replicates in the oral cavity and transmits via saliva. KSHV is the etiological agent of aggressive forms of primary effusion lymphoma (PEL). The KSHV-encoded ORF45 is an immediate early protein vital for lytic reactivation and virus production. We recently demonstrated that ORF45 promotes expression of a key human Forkhead transcription factor called FOXQ1, which contributes to the sustained lytic KSHV infection. It is still unclear how ORF45 can promote expression of key genes to drive lytic virus production. We aim to identify novel gene regulatory partners of ORF45, which might be used by ORF45 to promote viral reactivation.

Methods: Coimmunoprecipitation was used to identify novel partners of ORF45. Immunofluorescence assay using confocal microscopy revealed their subcellular co-localization. We mutated their interaction sites and used shRNA/siRNA to evaluate their role.

Results: Following up on proteomics studies, our co-immunoprecipitation and imaging analysis during lytic reactivation demonstrated that ORF45 interacts with host transcription factors, FOXK1 and FOXK2 and co-localizes with them in the nucleus of PEL cells. FOXK1 and FOXK2 are unique as they are the only Forkhead proteins which carry a Forkhead-associated (FHA) domain. We hypothesized that ORF45 interacts with these FOXK1 and FOXK2 through their FHA domain in order to promote the KSHV lytic cycle. FHA domains are specialized protein-protein interaction domains that bind to phosphorylated threonine motifs (pT) facilitating key functional interplays, but as of now no KSHV protein have been found to contain a functional pT motif. Through protein sequence alignment of known pT motifs with ORF45, we found a canonical pT motif within ORF45. Single amino acid point mutation of the pT in ORF45 abrogated ORF45-FOXK1/2 interaction. Our data points to a pro-viral role for the FOXK proteins as knockdown of FOXK proteins reduced lytic reactivation and infectious virus production in multiple KSHV positive cell lines.

Conclusions: FOXK proteins are constitutively expressed key members of the Forkhead transcription

Poster Presentation

Division

PhD/Post-doc

Abstract Title

Characterization of a Novel Zinc Export System in Streptococcus mutans

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Authors

A. M. Peterson*, T. Ganguly, J. Lemos

Area of Interest

Basic Science Research

Abstract

Objectives: Zinc has a long history as a therapeutic agent and is incorporated to oral care products to prevent or aid in the treatment of gingivitis, halitosis, and, arguably, control dental plaque buildup. Yet, the impact of high zinc levels on the oral microbiome is poorly understood. Recently, we showed that Streptococcus mutans, a major pathogen of dental caries, is more tolerant to zinc intoxication than other streptococci. This heightened tolerance was linked to a novel zinc exporter (ZccE) and cognate regulator (ZccR) that are unique to S. mutans. Therefore, ZccE and ZccR are potential targets for the development of species-specific therapeutic modalities. This study aimed to uncover the ZccR regulon and elucidate the mechanisms of ZccR regulation.

Methods: The ZccR regulon was determined by comparing transcriptomes of parent (UA159) and Δ zccR via RNAsequencing. Bioinformatics, EMSAs, bacterial two-hybrid (BTH) system and protein modeling were used to investigate ZccR-DNA interactions, search for ZccR-binding motifs, and predict oligomer formation. Results: Comparison of UA159 and Δ zccR transcriptomes revealed 150 differentially expressed genes, with an overrepresentation of genes related to sugar uptake. Growth curves revealed that Δ zccR grows poorly in most single sugar sources, with these growth defects exacerbated under sub-inhibitory zinc concentrations but rescued by manganese supplementation. EMSAs revealed that ZccR specifically binds to the zccE-zccR intergenic region (IGR) in a zinc-dependent manner, with preliminary evidence pointing to a palindromic sequence within this IGR as critical for this interaction. Protein modeling and BTH indicate that ZccR forms homodimers in the presence of zinc.

Conclusions: Beyond zccE, ZccR influences transcription of various other genes, particularly those related to sugar utilization. Further investigation is needed to determine the direct or indirect nature of these effects. Bioinformatics and molecular analysis suggest that ZccR forms homodimers in the presence of zinc to recognize its DNA-binding motifs.

Additional Information

The abstract lost its italicization

family, but their role in oncogenic virus production is unknown. We demonstrated that FOXK proteins interact with the viral protein ORF45 through a novel motif and promote infectious KSHV production.

Poster Presentation

Division

PhD/Post-doc

Abstract Title

Conserved Amino Acid Supports Forkhead Domain Interactions with Non-Homologous End-Joining Pathway

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Area of Interest

Basic Science Research

Abstract

Objectives: Kaposi's sarcoma-associated herpesvirus (KSHV) is an oncogenic herpesvirus that replicates in oral epithelial cells and spreads via saliva. Our lab revealed that host transcription factor FOXQ1 is induced following KSHV infection of oral epithelial cells and sustains lytic infection. FOXQ1 belongs to the Forkhead box (FOX) family of transcription factors that share a conserved DNA binding domain called the forkhead domain. Four FOX factors (including FOXL2) were shown to interact with the Non-Homologous End-Joining pathway (NHEJ) protein Ku70. The Ku70/Ku80 heterodimer together with ILF2/ILF3 is critical in the NHEJ pathway. FOXL2 binds to Ku70/Ku80 via the forkhead domain and disrupts this heterodimer. We hypothesize that FOXQ1 promotes lytic infection in oral epithelial cells by disrupting NHEJ complex assembly thereby blocking its known antiviral activity.

Methods: We performed immunoprecipitations in HEK 293T cells to test interaction. Since the interaction of FOXQ1 with key co-factors depends on a specific amino acid in the forkhead domain, we disrupted this amino acid in FOXQ1 and evaluated its impact on the interaction with NHEJ proteins.

Results: Interaction of FOXQ1 with Ku70/80 and ILF2/ILF3 was detected. The forkhead domain point mutation eliminated interaction between FOXQ1 and Ku70/Ku80, and reduced interaction between FOXQ1 and ILF3. FOXQ1 overexpression reduced interaction between Ku70 and ILF3.

Conclusion: FOXQ1 interacts with Ku70/80 and ILF2/ILF3 via the forkhead domain and might block NHEJ function. FOXQ1 uses a specific amino acid for this interaction, and our comprehensive sequence analysis of the Forkhead family reveals that virtually all forkhead proteins possess the same type of amino acid at this site. Further analysis of the forkhead family will unveil the likely shared role of forkhead proteins regulating NHEJ functions. Our future studies warrant a systematic evaluation of whether all forkhead proteins disrupt NHEJ complex antiviral function thereby supporting lytic viral replication in the oral cavity.

Poster Presentation

Division

PhD/Post-doc

Abstract Title

Deletion of any subunit of The Main Carbohydrate Transporter in Oral Streptococcus can Increase Cellular Fitness

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Area of Interest

Basic Science Research

Abstract

Objective: Metabolism of oral streptococci is heavily influenced by the concentrations of available sugars in the environment, a phenomenon referred to as carbon catabolite repression (CCR). Glucose is transported into the cell primarily through the glucose-phosphoenolpyruvate::sugar phosphotransferase system (glucose-PTS), and its subsequent metabolism triggers CCR in the oral commensal Streptococcus sanguinis. Previous work unveiled the interconnection of the sugar phosphorylating subunit, ManL, of the glucose-PTS with the main regulator of CCR, catabolite control protein A (CcpA). Here, we observed a single nucleotide polymorphism in a transmembrane subunit, ManN of glucose-PTS, that displayed altered organic acid excretion and increased H2O2 excretion in tandem with elevated PTS activity in multiple carbohydrates. We then sought to further understand the contributions of each component of the glucose-PTS to bacterial fitness.

Methods: we performed genetic analyses by deleting from the S. sanguinis lab strain SK36 the entire operon (EIIABCDOMan, generating strain Δ manLMNO), each PTS subunit (Δ EIIA, Δ EIIB, Δ manM, and Δ manN), and the uncharacterized manO (Δ manO), as well as CcpA (Δ ccpA) and the redox regulator Rex (Δ rex) for comparison. Results: Glucose-PTS subunit deletions incurred a primarily H2O2-mediated growth defect on glucose; when supplemented with catalase, these mutants produced a significantly higher yield and a doubling time comparable to the parental strain. H2O2 excretion was enhanced in all glucose-PTS deletions, leading to increased antagonism of the oral pathobiont Streptococcus mutans, a phenotype not reflected in the Δ ccpA mutant despite similarly increased H2O2 output. A clear shift in the pyruvate node towards mixed acid fermentation, paired with increased activity of the arginine deiminase system led to improved pH homeostasis in glucose-PTS mutants, but not the Δ ccpA mutant. Δ rex had no significant phenotype in most of the assays performed thus far. Conclusion: Taken together, alteration of any glucose-PTS subunit leads to a shift in strategy toward mixed acid fermentation and H2O2 production, resulting in increased persistence, better pH homeostasis, and increased

antagonism of sensitive species in a manner not dependent on the metabolic regulators CcpA and Rex.

Poster Presentation

Division

PhD/Post-doc

Abstract Title

Investigation of Extracellular Vesicles Composition and Function Within Periodontal Disease.

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Authors

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Area of Interest

Basic Science Research

Abstract

Objectives: Periodontal diseases are a class of chronic inflammatory gum diseases caused by bacterial infection that leads to destruction of alveolar bone and consequent tooth loss. There is evidence that oral health affects the overall health of patients by increasing risk for other systemic diseases, however the mechanism that links periodontal disease and systemic diseases is unknown. Extracellular Vesicles (EVs) have emerged as regulators of immune function and have been implicated in various disease and etiologies. We hypothesize that EVs act as an unidentified reservoir of immune modulators contributing to the immune dysfunction within periodontal disease and further exacerbate systemic inflammation, leading to the pathogenesis of other systemic diseases.

Methods: To test this hypothesis, EVs were isolated using differential centrifugation from both saliva and plasma from adult patients that were highly prone (high responders) and highly resistant (low responders) to gingivitis. EVs size and concentration was analyzed using Nanoparticle Tracking Analysis (NTA). EVs were lysed and soluble cyto/chemokines and DAMPS (e.g., IFN-gamma, IL-1ß, IL-2, IL-6, IL-10, IL-12p70, MCP-1, and TNF-a) were quantified using Luminex multiplex assay. For in-vitro EV transfers, 5x105 THP-1 cells were plated in a 24-well cell and stimulated with 200nM phorbol 12-myristate-13 acetate (PMA) for 24 hours. Cells were exposed to 3x107 EVs in the absence or presence of LPS from Escherichia coli O11:B4 for 24 hours. Supernatants and cellular mRNA were harvested for analysis. Results: NTA of saliva and plasma resulted in no significant difference in concentration of EVs, however when groups were stratified by High Responders vs Low Responders, there were differences in the composition of EVs (p=value <0.0001). We observed no significant differences in concentrations of cyto/chemokines from EV lysis. In-vitro EV transfer in the absence of LPS resulted in increased concentrations (pg/mL) of IL-1ß, IL-6, IFN-gamma, TNF- a and MCP-1, however these differences were not significant between groups.

Conclusions: Together these findings suggest that EVs contain immunomodulators and are immunostimulatory toward macrophages. Future works are looking to understand the effect of EVs on other cells within the oral cavity.



UF College of Dentistry UNIVERSITY of FLORIDA

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FACULTY/STAFF

ABSTRACT PRESENTATIONS







Poster Presentation

Division

Faculty/Staff

Abstract Title

Characterization of Interactions among Streptococcus mutans Protein Translocation Machinery Components

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Area of Interest

Basic Science Research

Abstract

Objectives: Most virulence attributes of the cariogenic Gram-positive pathogen Streptococcus mutans, including acidogenicity, aciduricity, biofilm formation, and localization of adhesins, are enabled by membrane proteins. Characterization of protein translocation machineries is essential to understanding S. mutans' pathogenicity. Membrane protein transport involves post- and co-translational processes and complex multi-component assemblies. For co-translational transport, the hydrophobic N-terminus of nascent polypeptide substrates is recognized by the signal recognition particle (SRP) comprised of a GTPase protein, Ffh, a small cytoplasmic RNA (scRNA), and sometimes an accessory protein YIxM. The SRP-ribosome nascent chain (RNC) complex is targeted to another GTPase, the membrane-localized SRP receptor FtsY. FtsY interacts with the SecYEG translocon channel to enable protein transport into or thru the membrane bilayer. YajC is an accessory translocon component. FtsY and Ffh binding results in GTP hydrolysis, enabling recycling of SRP pathway components and dockage of the translating ribosome to Sec translocon. Bacteria also contain one or more paralogs of the chaperone/insertase YidC that facilitates membrane protein translocation by both Sec-dependent and -independent mechanisms. Gram+ bacteria contain two YidC paralogs, and YIxM as part of the SRP. We aimed to identify interactions between specific proteins using the Bacterial Two-Hybrid System (BACTH) to gain insight into cooperative membrane protein translocation pathways.

Methods: We cloned genes encoding yidC1, yidC2, secYEG, ffh, ftsY, ylxM, and yajC into BACTH plasmids for expression as N- or C-terminal fusion partners of the two components of adenylate cyclase. Upon interaction of test proteins, adenylate cyclase components come together to reconstitute a functional enzyme. A positive interaction is visualized by formation of blue colonies on X-Gal indicator plates.

Results: We confirmed previously known interactions, i.e Ffh-FtsY, and identified novel protein-protein interactions, including YIxM dimer formation. We also observed that interactions among smaller proteins were better visualized as N-terminal rather than C-terminal fusions with adenylate cyclase. Conclusions: BACTH is a facile valuable tool to screen for individual protein-protein interactions among

numerous components within multi-protein complexes enabling dissection of protein translocation pathways in S. mutans.

Additional Information

It would not let me italicize any of the gene or organism names.

Poster Presentation

Division

Faculty/Staff

Abstract Title

Mitochondrial dsRNAs Activating PKR Alters Innate Immune Functions of SUV3-dificient Monocytes in Childhood Sjögren's Disease

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Area of Interest

Basic Science Research

Abstract

Objectives:

Childhood Sjögren's disease (cSjD) is a rare condition characterized by frequent recurrent parotitis. Our recent scRNA-seq analysis of PBMC from cSjD patients revealed the downregulation of Suv3-like RNA helicase (SUV3) in monocytes along with upregulated IFN-related genes compared to healthy controls (HC). SUV3 complexed with polynucleotide phosphorylase in degradomes unwinds RNA secondary structures in the mitochondria for RNA decay. This study aims to explore the roles of SUV3 in the innate immunity of cSjD monocytes.

Methods: scRNA-seq compared the transcriptomic profiles of cSjD and HC PBMC samples (n=4/group). To simulate SUV3 downregulation in vitro, cells were transfected with SUV3 shRNA or siRNA. Double-stranded RNAs (dsRNAs) were screened in patient monocytes by J2 anti-dsRNA antibody and the presence of mitochondrial dsRNAs (mtdsRNA) was detected by transmission-electron-microscopy (TEM) and RT-qPCR. Formaldehyde crosslinking immunoprecipitation (PKR-fCLIP)-RT-qPCR was applied to SUV3 KD THP-1 cells to determine mtdsRNA binding to a cytoplasmic dsRNA sensor, protein kinase R (PKR), and the levels of interferon-stimulating-genes (ISGs). Monocyte/macrophage functions such as ATP synthesis, superoxide production, phagocytosis, and migration were also analyzed.

Results: SUV3 knockdown (KD) led to cytoplasmic dsRNA accumulation and elevated IFN signature gene expression in vitro. When the cells were pre-incubated with actinomycin D, a mitochondrial transcription inhibitor, the J2 signal was abolished, indicating that dsRNAs originated from the mitochondria. TEM captured the presence of dsRNAs in the mitochondrial-derived vesicles of patient monocytes. Based on concomitant PKR upregulation in our sequencing data, PKR-bound RNA species in SUV3KD THP-1 cells were analyzed by RT-qPCR of mitochondrial transcripts, which confirmed mtdsRNA binding to PKR. Macrophages differentiated from SUV3 KD THP-1 cells or primary monocytes downregulated ATP synthesis and phagocytic activity, upregulated superoxide production, and decreased migration in M0 or M1 polarized macrophages, resulting in altered monocytes and

macrophage functions.

Conclusion: Deficient SUV3 expression in stable or primary monocytes induces mtdsRNA production and PKR activation, potentially contributing to aberrant innate immune functions with elevated IFN signature genes observed in cSjD monocytes.

2024 SPRING SYNERGY SUBMISSION FORM

Division

Faculty/Staff

Abstract Title

Role of Inflammatory Receptor TLR4 in Induction of Alveolar Bone Resorption after Ecological Time-sequential Polybacterial Periodontal Infection in TLR4-/- Mice.

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Authors

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Abstract

INTRODUCTION: Periodontitis is a polymicrobial dysbiotic chronic inflammatory disease caused by microbes interacting in the host subgingival sulcus/pockets. Streptococcus gordonii, Fusobacterium nucleatum, Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia are common co-colonizers of the supra- and subgingival sulcus/pockets in humans. The innate immune system has a broad range of specificity in detecting foreign antigens, and detection of infectious agents relies to a great extent on a family of evolutionarily conserved pattern recognition receptors (PRR), known as the Toll-like receptors (TLRs), which have a crucial role in early host defense against invading pathogens. Among several families of PRRs, TLRs are the bestcharacterized. TLR2 and TLR4 are located on the cell surface and are recruited to phagosomes after activation. OBJECTIVE: The purpose of this investigation was to determine alveolar bone resorption (ABR) after ecological time-sequential polybacterial periodontal infection (ETSPPI) with S. gordonii, F. nucleatum, P. gingivalis+T. denticola+T. forsythia in TLR4-/- mice. The aim was to demonstrate the role of inflammatory receptor TLR4 critical in ABR induced by polybacterial infection. METHODS: Ten-week-old male and female TLR4-/- mice (n=20) were used for polymicrobial ETSPPI infection (Group I) and sham-infection (Group II). Both maxilla (left and right) and mandible (left and right) were harvested after 17 weeks of (ETSPPI) infection. The right mandibles and maxilla were autoclaved for 20 min, and the defleshed jaws were immersed in 3% hydrogen peroxide for 30 min and air-dried. The teeth samples were then stained with 0.1% methylene blue. Two-dimensional molar teeth images were captured using a stereo dissecting microscope. The area between the cemento-enamel junction (CEJ) to the alveolar bone crest (ABC) of the buccal and the palatal surfaces of the maxillary and mandibular jaws was measured by using the line tool (AxioVision LE 29A software). RESULTS: TLR4-/- mice exposed to 17-week-polymicrobial infection significantly increased ABR (p<0.1) in the right maxillary buccal compare to sham infection. Right maxillary palatal of TLR4-/- mice have significantly (p<0.01) increased ABR and p<0.001 in the right mandibular buccal compare to sham infection. CONCLUSION: Multiple oral bacteria colonized on the gingival surface in the polybacterial TLR4-/- infected mice lead to significant ABR and periodontitis.

Poster Presentation

Division

Faculty/Staff

Abstract Title

Trivalent Immunization Using Metal-Binding Lipoproteins Confers Protection Against Enterococci in a Mouse Infection Model

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Area of Interest

Basic Science Research

Abstract

Objectives: To develop a broadly protective protein-based enterococcal vaccine using Enterococcus faecalis zinc- (AdcA, AdcAII) and manganese-binding lipoproteins (EfaA).

Methods: Full-length EfaA, AdcA, and AdcAII were expressed using the XpressCF[™] cell-free system (CFPS). Mass spectrometry and gel electrophoresis was used to confirm the identity, purity, and stability of each protein. The recombinant proteins were then used to raise polyclonal antisera in rabbits. Specificity of rabbit polyclonal antibodies were validated via ELISA and immune-blotting. To test for neutrophil-mediated killing of E. faecalis, opsonophagocytic killing assay (OPA) was performed using single-antigen (EfaA, AdcA, or AdcAII-specific) or multi-antigen (EfaA+ AdcAII, or EfaA+AdcA+AdcAII-specific) antisera. Passive immunization using single- and multi-antigen antisera were performed using the Galleria mellonella invertebrate model and mouse peritonitis model. Using the same peritonitis model, active immunization was also performed using lethal and non-lethal E. faecalis infections, respectively.

Results: While both the single- and multi-antigen antisera have similar capacities in mediating neutrophilfacilitated killing of E. faecalis, including clinical isolates of E. faecium; we found that immunization with the single-antigen antisera had negligible effect in G. mellonella larvae survival, and negligible-tomoderate reduction in bacterial titers in the mouse peritonitis model. Notably, passive or active immunization using a multivalent approach (2-antigen (EfaA + AdcAII) or 3-antigen (EfaA + AdcA + AdcAII)) conferred significant protection against enterococcal infections, including lethal infections of E. faecalis.

Conclusion: In totality, these findings indicate that a trivalent vaccine based on metal-binding lipoproteins elicits protection against enterococcal infections. While this is the first report showing feasibility of a multivalent approach, our findings serves as scaffold for the development of a broadly protective antienterococcal vaccine.

Poster Presentation

Division

Faculty/Staff

Abstract Title

Toward MR protocol-agnostic, unbiased brain age predicted from clinical-grade MRIs

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Area of Interest

Clinical Science Research

Abstract

The difference between the estimated brain age and the chronological age ('brain-PAD') could become a clinical biomarker. However, most brain age models were developed for research-grade high-resolution T1-weighted MRIs, limiting their applicability to clinical-grade MRIs from various protocols. We adopted a dual-transfer learning strategy to develop a model agnostic to modality, resolution, or slice orientation. We retrained a convolutional neural network (CNN) using 6281 clinical MRIs from 1559 patients, among 7 modalities and 8 scanner models. The CNN was trained to estimate brain age from synthetic research-grade magnetization-prepared rapid gradient-echo MRIs (MPRAGEs) generated by a 'super-resolution' method. The model failed with T2-weighted Gradient-Echo MRIs. The mean absolute error (MAE) was 5.86–8.59 years across the other modalities, still higher than for research-grade MRIs, but comparable between actual and synthetic MPRAGEs for some modalities. We modeled the "regression bias" in brain age, for its correction is crucial for providing unbiased summary statistics of brain age or for personalized brain age-based biomarkers. The bias model was generalizable as its correction eliminated any correlation between brain-PAD and chronological age in new samples. Brain-PAD was reliable across modalities. We demonstrate the feasibility of brain age predictions from arbitrary clinical-grade MRIs, thereby contributing to personalized medicine.