List of Supervisors and Projects for Summer Research Program 2017

1. Dr. Leslie Laing

**Project Title:** Effect of Edible Oils on Dental Erosion

**Project description:** As a result of decreased salivation, patients with Sjögren’s Syndrome (SS) frequently experience rampant dental decay, gingivitis, and candidiasis. It is of vital importance that agents be identified that might protect the tooth surface from demineralization since the natural oral buffer capacity of saliva is reduced (1, 2). Lipids have been shown to inhibit carious demineralization by providing a diffusion barrier within the organic protein-lipid-water matrix of enamel which may decelerate caries demineralization. Topically applied olive oil emulsions have been shown to be slightly effective in reducing artificial dentin caries lesions (3). Individual compositional differences among lipids may affect the attachment of the salivary pellicle to dental hard tissues (4) since the lipid content of the pellicle contributes to its ability to retard acid diffusion. We have recently confirmed the oral health benefits of the ancient Ayurvedic technique of oil-pulling with Virgin Coconut Oil (VCO) in SS patients whereby counts of *Strep. mutans* and *Candida sp.* were decreased by up to 100-fold. In an attempt to understand the mechanism behind the reduction of the microbial populations, the **objective** of our project is to determine whether edible oils are effective in reducing enamel or dentin demineralization, with the **hypothesis** that VCO will have the greatest effect. **Experimental Plan:** Bovine and human permanent incisors stored in 0.5% thymol solution will be separated into cementum, enamel, and dentin by grinding and polishing with water-cooled carborundum paper (800-, 2400-, and 4000-grit, respectively) resulting in plane surfaces approximately 200 µm thick. Baseline weights of 10 samples of each specimen type will be measured using a high-precision scale. Specimens will be submitted to 10 alternating demineralization and remineralization cycles, each including 5 min pretreatment with one of the test agents: distilled water (negative control), VCO, olive oil, sesame oil, safflower oil, or 13.2 mmol.l⁻¹ (250 ppm) fluoride solution pH 3.88 (positive control). Samples will be rinsed in tap water, transferred to artificial saliva (5) for 30 min, demineralized in 1% citric acid (pH 2.3) for 3 min, rinsed in tap water and again transferred to artificial saliva for 60 min. This cycle (5 min pretreatment, 30 min artificial saliva, 3 min erosion, 60 min artificial saliva) will be repeated 10 times. Loss of cementum, enamel, and dentin due to erosion will be determined by comparison to baseline measurements.

**References:**


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2. Dr. Limor Avivi-Arber

Project Title: The Transparent Brain - Visualizing Brain Changes Induced by Endodontic Treatment Versus Tooth

Project description:

OBJECTIVE: To use rat models of endodontic treatment and tooth extraction and utilize the novel CLARITY technique to quantify differences in the effects of tooth extraction versus endodontic treatment in the numbers and morphological features of immunostained neurons and non-neuronal astroglial cells within the orofacial sensorimotor cortex (OSMCx), a brain region involved in processing and controlling oral sensory and motor functions.

HYPOTHESIS: Tooth extraction as compared with endodontic treatment will induce a significantly larger changes in the number and morphological changes of neurons and astroglial cells in the oSMCx.

RATIONALE: When treating your patients, how aware are you that these treatments can differentially change the structure and function of your patient's brain? Such changes may underlie your patients' ability to adapt (or not) their oral sensory and motor functions to the treatment you provide them. For understanding brain mechanisms underlying oral sensory-motor functional adaptation, it is important to understand the treatment-induced cellular changes that occur in the oSMCx. CLARITY is a novel method that allows for immunostaining and three-dimensional imaging of cellular structures within thick brain specimens (e.g., a whole brain).

METHODS: Under general and local anaesthesia, adult male Sprague Dawley rats will receive either pulpectomy or extraction or sham operation of the three right maxillary molar teeth (n=8). A naïve group (n=8) will receive no treatment. Rats will be perfused on postoperative day 1, 7 and 28. The brains will be removed and go through a clearing process and immunostaining of 1 mm-thick brain tissue that includes the oSMCx. Immunostaining for GFAP (astroglia), and NeuN (neurons) will be used. Specimens will be scanned with a light sheet fluorescence microscope. For quantitative assessment of immunofluorescence stained cells, selected regions within the orofacial sensorimotor cortex will be identified, measured, and analyzed using Bitplane Imaris software. The number of neurons and astroglia and their morphological features will be determined and compared across animals using ANOVA and post-hoc testing as appropriate. The summer student will assist the graduate student with the dental procedures, brain tissue clearing and immunostaining, and will be mainly responsible for scanning and analysing the brain tissues.

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3. Dr. Karina Carneiro

**Project Title:** DNA nanostructures as templates for biomineralization

**Project description:**

Mineralized tissues, such as teeth and bone, form through a multi-step process guided by a complex mixture of proteins secreted by specialized cells. The exact mechanism in which the proteins template molecules to become highly organized and eventually transform into a mineralized tissue is challenging to decipher because of the ever changing composition of the matrix solution. A better understanding of mineralized tissue formation is essential for the development of regeneration strategies.

The hypothesis of this project is that the precise arrangement of peptides and proteins, known to promote hydroxyapatite formation, onto a surface will yield a material capable of nucleating and controlling crystal growth in a predictable manner. One approach to organizing groups with nanometer precision is to use synthetic DNA lattices, as has been demonstrated in the field of DNA nanotechnology. The objective of this project is to establish structure-properties relationships for DNA-amelotin surfaces as templates for nanostructured hydroxyapatite. The experimental plan is:

1) To couple DNA strands to peptides and proteins known to induce mineral formation;
2) To incorporate these DNA-conjugates into previously established DNA lattices;
3) To expose these functionalized DNA lattices to mineralizing conditions.

A successful completion of this project will elucidate how the underlying arrangement of mineralizing groups influence crystal nucleation and growth. This knowledge is necessary for the design of regeneration strategies for mineralized tissues. This project will be co-supervised by Prof. Carneiro and Prof. Ganss.

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4. **Dr. Iacopo Cioffi**

**Project Title #1:** Jaw muscle abnormalities in patients affected with chronic masticatory muscle pain and temporomandibular disorders

**Project description:**

Temporomandibular disorders (TMD) include a set of pathological conditions of the temporomandibular joints and the muscles of mastication. TMD are associated with facial pain, temporomandibular joint clicking, soreness and fatigue of the jaw muscles and masticatory dysfunction, thus impairing significantly the quality of life.

The masseter is one muscle of mastication involved in many physiologic jaw functions. It is also strongly active during parafinctional activities that are frequently found in individuals with TMD. This muscle has a pennate architecture, including robust aponeuroses (i.e. layers of fibrous tissue) delineating discrete muscle compartments. Such architecture represents an anatomical counterpart of functional heterogeneity. During jaw functioning, masseter muscle sub-portions can be differentially activated, ensuring a better performance, reducing the risk of muscle overloading and tissue damage, and allowing for a proper response of the muscle in case of high functional demand. Also, aponeuroses contribute to the storage of elastic energy and to protect muscle fibers from contraction-induced or high-strain injuries. Abnormalities in the aponeurosis content as well as in the arrangement of compartments may be associated to the onset of TMD.

In this study, the student will compare the architecture of the masseter in patients with muscular TMD and healthy controls to test whether the three-dimensional arrangement of muscle compartments and aponeuroses are abnormal in TMD patients. It is hypothesized that TMD patients will have fewer aponeurosis content and different distribution of aponeuroses from healthy controls.

The magnetic resonance imaging scans of 17 women with muscular TMD recruited at Mount Sinai Hospital and 17 healthy pain-free controls will be analyzed with a software. The outlines of the masseter muscle and muscle aponeuroses will be identified with an automatic built-in segmentation tool and then fine-tuned by manual contour tracing. The volumes of the segmented muscle compartments and aponeuroses will then be calculated and compared. Each muscle will be also subdivided into eight regions of interest to assess the 3D distribution of muscle compartments. This study will provide new knowledge about the structural abnormalities of the masseter muscle in individuals with TMD, thus contributing to a better understanding of the etiology of this condition.

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Project Title #2: Blood oxygenation of the masseter muscle in individuals with high vs low frequency of oral parafunctional behaviors

Project description:

Oral parafunctional behaviors, such as tooth clenching, are activities which go beyond physiological functioning, such as chewing, which contribute to the onset of temporomandibular disorders (TMD). Parafunctional tooth clenching determines intramuscular hypoxia, pain and fatigue of the jaw muscles and the onset of TMD-like symptoms in healthy volunteers. Also, disturbances of jaw muscles blood flow, determining intramuscular hypoxia, are present in individuals with TMD of muscular origin. Hence, it is conceivable that abnormalities in jaw muscle oxygenation may be contributing factors for the onset of TMD.

This study aims to assess whether individuals with frequent self-reports of daytime clenching episodes, who are known to be at greater risk for TMD, present abnormalities in jaw muscles oxygenation as compared to individuals with low frequent clenching episodes. It is hypothesized that there will be significant differences in muscle oxygenation between the study samples.

The oral behaviors checklist, an instrument able to detect the occurrence of waking-state parafunctional behaviors, was submitted to Students on St. George Campus to select two groups (>80th percentile and <20th percentile of the frequency distribution, i.e. high parafunctional group-HP, and low parafunctional group-LP). The activity of the right masseter, and oxyhemoglobin, deoxyhemoglobin, total hemoglobin concentrations and oxygen saturation will be monitored by means of surface electromyography and near infrared spectroscopy during three standardized experimental sessions separated by two days intervals. In the first session, baseline recording (rest) will be collected for 30 minutes. Thereafter, participants will chew a chewing-gum for a maximum of 30 minutes at a normal rate (1.5 Hz). In the second session, they will keep their teeth in contact at 10% of their maximum voluntary contraction (MVC). In the third session, they will do the same but at 40%MVC. The order of the sessions will be randomized. Pain and fatigue will be collected on an electronic visual analogue scale every minute. Between groups and between sessions differences in oxyhemoglobin, deoxyhemoglobin, total hemoglobin concentrations, oxygen saturation, and muscular activity over time will be analyzed. This study will provide novel information about the functional abnormalities of the muscles of mastication of individuals at greater risk of TMD.

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6. Dr. Yoav Finer

**Project Title:** DEGRADATIVE ACTIVITIES FROM HUMAN NEUTROPHILS TOWARD DENTAL COMPOSITES, TOOTH DENTIN AND THE RESTORATION-TOOTH INTERFACE

**Project description:**

**Background:** Methacrylate-based polymeric resin composite are the most popular dental restorative materials. However, the ester groups of their polymer networks are susceptible to hydrolysis by salivary and bacterial esterases. Collagen is the major component in dentin organic matrix and is degraded by protease activity, either the dentin’s matrix metalloproteinases (MMPs) or from bacteria. These degradative activities adversely affect these materials and the material-tooth interface, accelerating the premature failure of the restoration. In the oral cavity, dental restorations are continuously exposed to neutrophils, hypothesized to have and contain esterases and collagenases activities that could enhance degradation of the materials and the restoration-tooth interface.

**Rationale:** Elucidating host-biomaterial interactions would allow for the development of materials and techniques to reduce interfacial degradation.

**Hypothesis:** Human blood neutrophils (HN) possess esterase and proteases activities that degrade the restoration, tooth-dentin and restoration-tooth interface.

**Objectives:**
1) Measure esterase, cholesterol esterase-like (CE-like) and pseudocholinesterase-like (PCE-like) activities of HN
2) Measure the effect of HN on the degradation of methacrylate resin composites, total-etch and self-etch adhesives
3) Measure protease, MMP-like activities from HN
4) Measure the degradative activity of HN toward human dentinal collagen

**Methods:** Freshly isolated HN (UofT Ethics Protocol #29410) will be tested for CE-like and PCE-like activities using nitrophenyl or butyrylthiocholine substrates, respectively. Protease activity in HN toward generic and specific human matrix metalloproteinases (MMP-1, -2, -8 and -9) will be measured using the SensoLyte MMP Assay Kit (AnaSpec). The ability of HN to degrade resin composite, total-etch and self-etch adhesives will be examined by quantifying the release into the incubation media of bishydroxypropoxy-phenyl-propane (BisHPPP), a universal resin degradation byproduct, using ultra high performance liquid chromatography (UPLC). The ability of HN to degrade the collagen in demineralized dentin will be tested by quantifying the amount of hydroxyproline released in the supernatant of incubation solutions and by observing the tissues under scanning electron microscopy (SEM). The degradative effect of HN on the interfacial bond strength of composite bonded to dentin will be measured using miniature short-rod resin-dentin fracture toughness specimens fabricated with resin composite and either total-etch or self-etch adhesives after exposure to HN.

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7. Dr. Boris Hinz

**Project Title #1: Attraction of Inflammatory Macrophages to Fibrotic Myofibroblasts is Dynamic**

**Project description:**

**Background and Rationale:** Chronic inflammation results in organ fibrosis. Responsible for detrimental contractures in tissue fibrosis are myofibroblasts (MFs), which secrete and contract collagen. Inflammatory macrophages (Mφ) are main producers of pro-fibrotic growth factors TGF-β1 that activates MFs. We found that physical contact with Mφ results in markedly increased TGF-β1 and MF activity but it is not known how proximity is initially established.

**Hypothesis:** We hypothesize that Mφ detect collagen displacements created by contracting MFs - analogous to an angler `feeling' the pulling fish on the other end of the fishing rod.

**Experimental Plan:** To obtain MFs, we will explant primary mouse dermal fibroblasts. Primary monocytes will be isolated from mouse bone marrow and culture-polarized into pro-fibrotic Mφ2 using Mφ colony stimulating forming factor (MCSF) and interleukin (IL)-4/IL-13. For `attraction assays', Mφ will be added to collagen gels and live recorded for 6 h. Image sequences will be analyzed for Mφ migration by automated tracking. Tracks will be analyzed for mean squared displacement, migration directionality, speed, and distance with relation to the force source (see below). MFs will be cultured on collagen gels to pre-organize collagen fibrils for 1 h before adding Mφ. To modulate the contraction activity of single MFs, we will use MFs transfected with an optogenetic construct (Rho-GEF) that, in brief, will induce MF contraction upon laser light excitation. We will investigate the effect of modulating light pulse (MF contraction) amplitude and frequency on Mφ attraction.

**Significance:** By dissecting the mechanisms of Mφ guidance to MFs, we aim to ultimately discover novel targets for anti-fibrosis strategies, such as elements of the Mφ mechanosensing and migration machinery.

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8. Dr. Boris Hinz

**Project Title #2:** Mechanical priming of mesenchymal stem cells for therapeutic tissue repair

**Project description:**

**Background and Rationale:** Hypertrophic scars are a tremendous burden for burn wound patients and are caused by myofibroblasts (MFs) that contract and remodel extracellular matrix (ECM). In contrast, mesenchymal stem cells (MSCs) have regenerative capacity but acquire MF characteristics when cultured on stiff surfaces for therapy expansion. We developed ‘skin-soft' substrate that suppress MF activation by imprinting epigenetic ‘mechanical memory'. Splinted rat wounds receiving ‘soft-primed' MSCs healed better compared with wounds treated with ‘scar-stiff'-primed MSC. It remains elusive how soft-primed MSCs exert this beneficial effect on wound healing.

**Hypothesis:** Delivered soft-primed MSCs will create a beneficial wound environment that prevents pathologic activation of endogenous repair cells.

**Experimental Plan:** We will use human umbilical cord perivascular MSCs (HUCPVs, collaboration JE Davies) which respond to soft-priming and escape xenograft rejection in a rat study. Rat wounds will be splinted with a plastic frame to prevent wound closure, exacerbate scarring, and create a reservoir to apply fluorescently labelled soft- and stiff-primed HUCPVCs. Animals will be sacrificed after 2-10 days of healing. Wound tissue will be harvested and analyzed for: (a) localization, and MF phenotype of engrafted MSCs; (b) Immune cell quantities, localization, types, and associated chemokine profiles (b) vascularization by staining for endothelial cell markers; (c) MF presence and persistence by assessing specific markers; (d) ECM composition, structure, and mechanical properties by confocal and second harmonics microscopy and micro-mechanical testing.

**Significance:** Establishing the timing and profile of inflammation, vascularization, differentiation of endogenous MFs, and neo-formation of ECM will help to understand which wound healing stages are affected by soft-primed MSCs.

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9. Dr. Celine Levesque

Project Title: Evaluation of Streptococcus salivarius isolates for use as oral probiotics

Project description:

Worldwide, 60-90% of school children and nearly 100% of adults have cavities or tooth decay. Caries is a chronic disease initiated by the presence of dental plaque biofilm that contains Mutans Streptococci (MS), a group of bacteria extensively characterized for its role in caries development and formation. Many therapeutics methods are being devised to specifically target bacteria in the biofilm without disturbing the microbiome. Significant among them is the use of probiotics which contain viable beneficial bacteria capable of producing a diverse range of antimicrobial peptides (bacteriocins). Several results demonstrated that many strains of Streptococcus salivarius, a resident of the normal flora of the mouth, are capable of producing a diverse range of bacteriocins, and have excellent potential for use as oral probiotics targeting MS. The aim of this project is to test clinical strains of S. salivarius isolated from caries-free children for inhibitory activity against MS. To accomplish our goal, we will pursue the following objectives: 1) Screening of S. salivarius isolates for production of bacteriocins active against clinical MS isolated from children with severe-early childhood caries; 2) Document safety data relating to potential probiotic S. salivarius isolates including assessment of antibiogram and virulence determinants; 3) PCR screening for the presence of structural genes encoding S. salivarius bacteriocins. This project will push the frontiers in the care and prevention of a hugely prevalent and costly worldwide infectious disease. The development of oral probiotic bacteria for the expression of bacteriocins against caries pathogens will lay the groundwork towards the development of new therapeutic chemoprophylactic molecules that could be further commercialized to be delivered as mouthwashes, toothpastes, gels and/or varnishes.

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10. **Dr. Marco Magalhaes**

**Project Title:** Three-dimensional assessment of invadopodia membrane composition

**Project description:**

Background: Currently, patients diagnosed with oral cancer have high mortality rates, primarily due to the local and distant spread of the disease at the time of diagnosis. Metastasis is driven by the ability of cancer cells to invade through interstitial tissues and basement membranes by forming actin-rich subcellular structures called invadopodia. Although some molecular mechanisms of invadopodia formation have been elucidated, initiating steps, including membrane composition have yet to be investigated in a 3D environment.

Objective: In this study, we investigate invadopodia phospholipid distribution changes at the plasma membrane during oral cancer cell invadopodia formation and maturation.

Methods: Oral cancer cell lines will be transfected with invadopodia markers Cortactin and Tks5 as well as fluorescent tagged phospholipid probes for PI(4,5)P2, PI3P (PtdIns3P) and PS. Single cells and spheroids will be analyzed in a 3D matrigel matrix using conventional confocal microscopy, light sheet microscopy and structured illumination.

Expected results: We will develop a method to evaluate invadopodia membrane composition in 3D and determine variations in the concentrations of PI(4,5)P2, PI3P (PtdIns3P) and PS.

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11.  Dr. Christopher McCulloch

Project Title: Mechanism of cyclosporin A-induced gingival overgrowth

Project description:

In high prevalence inflammatory diseases such as arthritis and periodontitis, excessive matrix degradation leads to loss of homeostasis and tissue destruction. In healthy periodontium, fibroblasts maintain connective tissue homeostasis by rapid, balanced synthesis and degradation of matrix proteins. In periodontitis, inflammatory cytokines drive uncontrolled matrix breakdown by fibroblasts that leads ultimately to tooth loss. Current treatment methods for periodontitis are expensive, time-consuming and are associated with significant morbidity.

Rationale: We identified a system that controls the remodeling and function of collagen fibrils in periodontal and dermal connective tissues. A key element of this system is filamin A (FLNA), an actin binding protein that regulates cell adhesion through interactions with integrins. We found that IL-1-induced collagen degradation mediated by the phagocytic and pericellular proteolytic pathways in fibroblasts required FLNA. While the molecular mechanisms by which FLNA controls collagen degradation are not well-defined, new insights into how FLNA controls matrix degradation may suggest that FLNA could provide a target for development of drugs that block destruction of connective tissues in inflammatory diseases.

Hypothesis: Interactions of FLNA with the beta 1 integrin are required for collagen degradation by phagocytosis and pericellular proteolysis by MMPs. Accordingly, introduction of cell permeable, FLNA peptides that mimic the interaction sites of FLNA with the beta 1 integrin in FLNA domain 21 and with protein kinase C in domain 20, will interfere with inflammation-induced destruction of the extracellular matrix protein, collagen.

Objectives: Develop a panel of cell-permeable FLNA peptides to enable specific interference of FLNA activation and its interactions with the beta 1 integrin. Evaluate the use of these peptides to block collagen degradation in cultured cells.

Experimental Plan: We will design and synthesize 10-15 mer peptides using the beta 1 integrin binding sequence in domain 21 of filamin A and the 15 mer peptide sequence surrounding the filamin A activation site, serine 2152 in FLNA. The peptides will include the cell-penetrating sequences derived from Tat to facilitate internalization We will treat cultured human gingival fibroblasts with these FLNA permeable peptides and assess their impact on FLNA activation, collagen phagocytosis and proteolysis that is induced by the inflammatory cytokine, IL-1.

Significance: An improved understanding of the molecular mechanisms by which FLNA controls matrix remodeling may suggest novel treatment strategies for clinical management of connective tissue degradation in inflammatory diseases such as arthritis and periodontitis. New treatment approaches based on cell-permeable, FLNA interfering peptides could help to reduce the costs and morbidity that is associated with current therapeutic approaches for periodontitis and could suggest alternative, local approaches for controlling matrix degradation in other inflammatory diseases of joints and skin.

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12. Dr. Tara Moriarty

**Project Title #1:** Role of hemolysin III in *B. burgdorferi*-induced inflammation in obese and non-obese mice

**Project description:**

Lyme disease, caused by the bacterium *Borrelia burgdorferi* (*Bb*), is a vector-borne infection. Its incidence is increasing in North America, analogous to the rising rate of obesity. Previous studies have demonstrated that *Bb* burden and *Bb*-induced carditis are aggravated in diet-induced obese mice fed a high-fat-diet (HFD). Moreover, we observed that *Bb* infection induces weight gain at a similar rate in mice fed a standard rodent chow (ND) as in HFD-fed mice. This suggests that *Bb* affects both the immune and metabolic function in mice.

Adiponectin is an anti-inflammatory regulator of metabolism and immune system. Biochemical analysis of a *Bb*-produced protein (annotated as hemolysin III) revealed that it may have an adiponectin-binding domain. Based on these results, we speculate that hemolysin III may be playing a crucial function in establishing *Bb* infection, possibly by binding host adiponectin. Hemolysin III and adiponectin interaction may be a plausible explanation for the association between Lyme disease and obesity as observed in the previous studies. If we detect defective infectivity in mutant *Bb* in this study, infection studies in adiponectin-deficient mice will be performed to further explore this relationship. The results from these studies may offer novel therapeutic targets for Lyme disease.

**Objectives:** To characterize inflammatory pathologies in obese and non-obese mice infected with Lyme disease pathogen *Borrelia burgdorferi* lacking hemolysin III (putative adiponectin-binding molecule).

**Hypothesis:** We hypothesize that hemolysin III plays an integral role in establishing Lyme disease infection; thus, mice infected with *Bb* strain lacking hemolysin III gene will lead to milder inflammation compared to wild-type *Bb*-induced inflammation. Moreover, we speculate that inflammatory pathologies will not be similar in obese and non-obese mice.

**Experimental Plan:** Mice have been preconditioned with ND or HFD for 12 weeks, followed by infection with *Bb* (Wild-type strain or strain lacking hemolysin III gene). Four weeks after the infection, the student will collect tissues and characterize Lyme carditis and arthritis using standard histological scoring methods. The student will also be collecting serum to quantify levels of adiponectin and other inflammatory cytokines.

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13. **Dr. Tara Moriarty**

**Project Title #2:** Determining the *Borrelia burgdorferi*-vascular adhesion mechanism in joint

**Project description:**

Lyme disease is caused by the bacterium *Borrelia burgdorferi*, and is the most common vector-borne infection in the developed world. Its incidence is increasing rapidly. A key step in disease progression is dissemination, which depends on *B. burgdorferi* adhesion to vascular endothelium and can result in arthritis. Pathogen-endothelial interactions depend on bacterial cell surface adhesion proteins (adhesins). Currently, only the adhesin BBK32 is known to support *B. burgdorferi* interactions with endothelia under physiological shear stress. Most pathogenic spirochetes, including *B. burgdorferi* are unusually invasive and can disseminate to and colonize most tissues in the body. There is great diversity in tissue-specific tropism patterns for many Lyme disease species and strains due to variations in the adhesins they produce. Our data suggest that *B. burgdorferi* encodes at least 5 additional vascular adhesins supporting *B. burgdorferi* dissemination to diverse tissues.

**OBJECTIVE:** Identify and investigate *B. burgdorferi* adhesins involved in interactions with joint endothelia under fluid shear stress. **HYPOTHESIS:** One of the 35 known and predicted adhesins identified in *B. burgdorferi* to date likely confers the ability to interact with endothelia in the microvasculature of joints. **PLAN:** We have developed a blood vessel-mimicking flow chamber system which allows for bacteria to be perfused at physiological shear stresses over primary human endothelia. The student will be using *B. burgdorferi* strains (either gain of function or knock outs for the 35 candidate vascular adhesins) expressing green fluorescent protein, or fluorescently labelled with live cell imaging membrane dye to identify adhesins that confer interactions with primary human endothelia derived from the joint microvasculature. All procedures required to complete this project are established in our lab. The student will be trained by two PhD students in the lab, who will assist her with bacterial and endothelial cultivation and train her in live cell imaging and analysis.

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14. Dr. Massieh Moayedi

**Project Title #1:** Elucidating the structural connectivity of the posterior insula and the mid-cingulate cortex in humans

**Project description:**

Pain is a fundamental sensory experience vital to an organism's survival. However, the mechanism of how pain is encoded remain unknown. Neuroimaging studies have identified a consistent pattern of brain activity when a subject feels pain. Recent evidence suggests that this activity encodes the salience content of a nociceptive stimulus, rather than the stimulus. Indeed, the patterns of brain activity elicited by innocuous and noxious somatosensory stimuli are indistinguishable using traditional univariate statistical approaches. Preliminary evidence from our group revealed that the dorsoposterior insula (dPINS) - a region activated in response to both innocuous and noxious somatosensory stimuli, revealed greater functional connectivity to the mid-cingulate cortex (MCC) in pain compared to iso-salient touch. Therefore, these data suggest that the dPINS-MCC may be specific to the perception of pain. However, this connectivity must be better characterized. One fundamental question is whether there is a structural substrate for this functional connectivity, or whether there are intermediate structures connecting these two regions. To answer this question, we will investigate the white matter connectivity of dPINS-MCC with diffusion weighted magnetic resonance imaging (dMRI). dMRI is a non-invasive technique to measure the diffusion of water molecules in the brain. White matter tracts contain myelin, a fatty sheath that wraps neurons, and restricts the diffusion of water. This anisotropic diffusion can be leveraged to model white matter pathways in the brain, a method known as tractography. This is particularly difficult between the insula and the MCC as there are several major white matter bundles that cross any connections that may exist. One method that overcomes such limitations is probabilistic tractography, which can delineate crossing fibres and identify likely tracts within the brain. The overall aim of this study is to elucidate the structural basis of dPINS-MCC connectivity using diffusion-based probabilistic tractography. We hypothesize that there will be some direct connections between the dPINS and the MCC. There will, however, also be connections via the mediodorsal thalamus, a common input to both regions.

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15. Dr. Massieh Moayedi

Project Title #2: Identifying pain-specific brain connectivity in humans

Project description:

Pain is a fundamental sensory experience vital to an organism's survival. However, the mechanism of how pain is encoded remain unknown. Neuroimaging studies have identified a consistent pattern of brain activity when a subject feels pain. Recent evidence suggests that this activity encodes the salience content of a nociceptive stimulus, rather than the stimulus. Indeed, the patterns of brain activity elicited by innocuous and noxious somatosensory stimuli are indistinguishable using traditional univariate statistical approaches. Preliminary data from our group investigating the connectivity of a region activated in response to both innocuous and noxious somatosensory stimuli - the dorsoposterior insula (dpINS) revealed a significantly stronger functional connectivity to the mid-cingulate cortex (MCC) during painful stimuli compared to iso-salient touch. Therefore, these data suggest that the dpINS-MCC may be specific to the perception of pain. However, this finding must be validated in an independent, larger sample. Indeed, the first aim of this study is to validate that dpINS-MCC connectivity is significantly stronger in pain than in iso-salient touch. Furthermore, given that pain is inherently unpleasant, it is possible that the connectivity encodes unpleasantness. Therefore, the second aim of this proposal is test the connectivity whether the connectivity encodes unpleasantness, rather than pain. We hypothesize that the connectivity will encode pain unpleasantness, but not unpleasant stimuli of other modalities. A third important question is whether this connectivity is intensity-dependent - does it become stronger with more intense painful stimuli. To test these hypotheses, we collected three fMRI datasets in an independent sample of subjects. In the first dataset (n=18), subjects received brief, intense painful stimuli and iso-salient brief, intense innocuous electric shocks. Subjects also provided trial-by-trial ratings for each of the stimuli. In the second dataset (n=18), subjects received 8s of iso-salient, unpleasant stimuli of three different modalities: pain, touch and visual images. We will use standard functional connectivity methods to investigate dpINS-MCC in both studies. Subjects also provided trial-by-trial ratings for each of the stimuli. In the third dataset (n=30), subjects received 4 different levels of painful stimuli, and provided trial-by-trial ratings.

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16. Dr. Dilani Senadheera

Project Title: Bacterial community correlations with refractory periodontitis, and discovery of novel disease indicator organisms

Project description:

BACKGROUND: Periodontal diseases are a group of polymicrobial inflammatory infections affecting tooth-supporting structures. Of the many forms, refractory periodontitis (RP) are the least characterized and medically perplexing. RP is defined as the continued degeneration of the periodontium despite adequate periodontal therapy and proper oral hygiene. Despite its multifactorial etiology suggesting that tissue breakdown is likely caused by an “overreacting” host immunity in response to a pathogenic oral microbial consortium, to date there are no studies that have attempted dissection of the comprehensive oral microbiome in these patients. Instead, many have focused on characterizing the oral bacterial communities associated with chronic periodontitis (CP), which is the leading cause of tooth loss, worldwide. Notably, CP-associated periopathogens including Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola (referred to as “Socransky’s Red complex pathogens) are commonly used as disease indicator species for treatment outcome surveillance when periodontal breakdown is suspected. Our preliminary real-time PCR-based plaque analysis suggests that RP is not correlated with typical CP-associated Red complex species (unpublished data). Here we ask: Are there unique oral bacterial fingerprints that can be identified in RP patients that are distinct from CP and health, which will help predict the risk for sustained periodontal breakdown?

HYPOTHESIS AND OBJECTIVES: We hypothesize that continued breakdown of the periodontium in RP patients (despite adequate therapy) are correlated with consistent OM shifts that are distinct from that in CP patients and healthy controls. Our goals are: 1) To understand how the oral microbiome associated with RP differs from CP and health; 2) to identify novel bacterial biomarkers for RP in multiple oral sites.

OUTCOMES: This is the first study that will comprehensively compare the oral plaque microbiome in RP, CP versus healthy controls. Results from this work will help identify novel oral bacterial biomarkers for tracking RP treatment-outcomes, which challenge the current approach of monitoring Socransky’s Red-complex periodontal pathogens in disease surveillance and treatment-outcome monitoring. Our results will create new knowledge in RP-prediction using machine learning to tailor preventive or treatment strategies with significant clinical, economic, and public health implications in Canada.

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17. Dr. Laura Tam

Project Title: Effect of tooth bleaching on human dentin fatigue resistance in vitro.

Project description:

Tooth bleaching is often seen by clinicians and patients as a safe and simple procedure. However, our data on bovine teeth indicate that their fatigue resistance can be significantly compromised by repeated use of bleaching agents (Tam et al. Effect of bleaching on fatigue resistance and flexural strength of bovine dentin. J Esthet Rest Dent 2015; 27:374 -382; Tam et al. Effect of whitening strips on fatigue resistance and flexural strength of bovine dentin. PLoS One 2017; in press). The fatigue resistance of human dentin when bleaching agents are applied has not yet been characterized.

The objective of this study will be to determine the effects of tooth bleaching on the fatigue resistance of human dentin. The null hypothesis will be: bleaching has no effect on the fatigue resistance of dentin. The in vitro study results will be later correlated to the results of an in situ study currently being developed by a MSc student, and also to our previous studies regarding the effect of bleaching on bovine dentin.

Methods

The dentin will be harvested from human molars and sectioned to form 1.2x1.2x12.0mm rectangular beam specimens. The specimens will be randomly divided into 2 treatment groups: control or bleach (Opalescence, Ultradent). A treatment session will consist of an application of treatment gel to dentin for seven hours. The specimens will be stored at 37°C, >80% relative humidity during treatment. Treatment sessions will be either daily for 2-weeks or 5-days-a-week for 8-weeks (n=10), to simulate typical and prolonged bleaching regimens. The specimens will be stored in 37°C artificial saliva between treatment sessions.

After the last treatment session, the dentin specimens will be mounted in custom-designed holders immersed in 37°C artificial saliva, and subjected to fatigue loading at 20MPa and 3 Hz for $10^6$ cycles using a chewing simulator (CS 4.4 Chewing Simulator, SD Mechatronik). Kaplan-Meier method with a log-rank test will be used to determine whether the time to failure differed. Wilcoxon test will be used for pairwise comparison. Cox regression will be used to assess the relationship between survival time and the covariates (treatment and time) (p<0.05).

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