List of Supervisors and Projects for Summer Research Program 2018

1. Dr. Yoav Finer

Project Title #1: DEGRADATIVE ACTIVITIES FROM HUMAN NEUTROPHILS TOWARD DENTAL RESIN ADHESIVES

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.

Impact: Improving the quality of the restorations and their ability to maintain their margins could enhance their longevity and reduce health and economic burdens.

Hypothesis: Human blood neutrophils (HN) possess esterase and proteases activities that degrade resin composite restorative material and adhesives.

Objective: Measure the effect of HN on the degradation of methacrylate-based total-etch and self- etch adhesives

Methods: Fresh HN (UofT Ethics Protocol #29410) will be isolated from human blood as described before. Blood will be layered onto 1-step polymorph and centrifuged, then HN will be collected, resuspended in 1mL of HBSS (Mg+/Ca+) buffer and stained with 0.4% trypan blue (1:1) to determine neutrophil cell number and viability prior to incubation. Freshly isolated HN will be then tested for CE-like and PCE-like activities using nitrophenyl or butyrylthiocholine substrates, respectively. **Degradation of resin adhesives by HN:** The ability of HN to degrade commercial resin total-etch (Scotch Bond MP) or self-etch (Easy Bond) adhesives (3M) will be studied by incubating with HN for up to 30-days, with replacement every 48 hrs to maintain activity in the incubation solutions. Measuring the release of bishydroxy-propoxy-phenyl-propane (BisHPPP), a universal resin degradation byproduct, via ultra high performance liquid chromatography (UPLC), will allow for quantification of degradation. Surface degradation of adhesive specimens will be done with scanning electron microscopy.

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2. Dr. Yoav Finer

Project Title #2: DEGRADATIVE ACTIVITIES FROM HUMAN NEUTROPHILS TOWARD 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.

Impact: Improving the quality of the restorations and their ability to maintain their margins could enhance their longevity and reduce health and economic burdens.

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

Objective: Measure the effects of HN on the interfacial bond strength of resin composite bonded to human tooth dentin

Methods: Fresh HN (UofT Ethics Protocol #29410) will be isolated from human blood as described before. Blood will be layered onto 1-step polymorph and centrifuged, then HN will be collected, resuspended in 1mL of HBSS (Mg+/Ca+) buffer and stained with 0.4% trypan blue (1:1) to determine neutrophil cell number and viability prior to incubation. **Interfacial bond strength:** The degradative effect of HN on the interfacial bond strength of composite bonded to dentin will be measured using miniature short-rod (mini-SR) resin-dentin fracture toughness specimens fabricated using resin composite bonded (Z250, 3M) to dentin with either total-etch (Scotch Bond MP) or self-etch (Easy Bond) adhesives (3M) after exposure to HN. Freshly isolated HN will be will be incubated with the mini-SR specimens. Cells will be replaced with fresh batch every 48 hrs based on viability and activity assays previously conducted. At the end of each incubation period specimens will be tested in a Universal Testing Machine (Model 8501, Instron®, Canton, MA, USA) and loaded in tension at an extension rate of 0.5 mm/min until fracture. Interfacial fracture toughness will be then calculated.

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3. Dr. Yoav Finer

Project Title #3: IDENTIFICATION OF DEGRADATIVE PROTEINS FROM HUMAN NEUTROPHILS THAT DEGRADE RESTORATIVE DENTAL MATERIALS AND TOOTH DENTIN

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 degradative that are active toward dental restoration and tooth dentin. **Impact:** Improving the quality of the restorations and their ability to maintain their margins could enhance their longevity and reduce health and economic burdens.

Objective: Identify proteins from HNs that are responsible for resin and dentin degradation and identify changes, in protein expression in response to incubation with resin and dentin

Methods: Fresh HN (UofT Ethics Protocol #29410) will be isolated from human blood as described before. Blood will be layered onto 1-step polymorph and centrifuged, then HN will be collected, resuspended in 1mL of HBSS (Mg+/Ca+) buffer and stained with 0.4% trypan blue (1:1) to determine neutrophil cell number and viability prior to incubation. Freshly isolated HN will be then 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). **Proteomic Analysis:** HN proteins will be identified using liquid chromatography tandem mass spectrometry (LC-MSMS) with the Xevo G2-XS QTof spectrometer (Waters Corporation, Mississauga, ON). HN will be incubated with or without resin and dentin and subject to rotary evaporation and rehydration. Sample solutions will be separated with a C18 column on a 100 minute reverse phase gradient, then run through electrospray ionization and scanned at a range of 390-2000 m/z. MSMS data will be queried with human protein databases Swiss Prot and TrEMBL and filtered and identified using the SEQUEST algorithm in Proteome Discoverer 1.3 software.

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4. Dr. Marco Magalhaes and Dr. Karina Carneiro

Project Title: Determining in *vitro* assembly of Cortactin-Tks5 complexes using atomic force microscopy (AFM)

Project description:

Rationale: Invadopodia are specific cancer cell protrusions that allow tissue penetration by concentrating matrix-degrading activity to contact points between cell and matrix. They represent sites in which cell signaling, proteolytic, adhesive, and cytoskeletal pathways physically converge. Invadopodia were identified in a number of invasive cancer cell lines, such as breast, head and neck, prostate, fibrosarcoma, and melanoma and recent evidence demonstrates molecular links between invadopodia and metastasis in mouse models and human patients. The precise relationship of core invadopodia components (e.g. Cortactin and Tks5) and their potential assembly in protein complexes is currently unknown. Atomic force microscopy (AFM) allows proteins to be imaged with resolutions of up to 0.1 nm, allowing us to observe single molecule events and study function and assembly of single proteins.

Hypothesis: Cortactin and Tks5 assemble in a protein complex that promotes elongation of invadopodia

Objetives: To determine the assembly of core invadopodia components Cortactin and Tks5 using AFM

Experimental plan: Purified Cortacin and Tks5 will be assembled alone and in combination under physiological conditions and imaged by AFM in ambient and fluid modes. We will evaluate the molecular interactions of these proteins with a goal towards a better understanding of the mechanism of invadopodia formation in oral cancer *in vivo*.

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5. Dr. Christopher McCulloch

Project Title: Role of Filamin A in progression of periodontitis

Project description:

Background: 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.

Rationale: We identified a system that controls the remodelling and function of collagen fibrils in periodontal and dermal connective tissues. A key element of this system is filamin A, 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 filamin A. We synthesized cell permeable peptides that compete for filamin A binding sites and discovered that these peptides block IL-1-induced collagen degradation. While the molecular mechanisms by which filamin A controls collagen degradation are not well-defined, new insights into how filamin A controls matrix degradation may suggest that filamin A could provide a target for development of drugs that block destruction of connective tissues in inflammatory diseases.

Hypothesis: Interaction of filamin A with the 1 integrin is required for cytokine-induced collagen degradation by phagocytosis and pericellular proteolysis.

Specific Aims: Assess how the pro-inflammatory cytokine IL-1 affects filamin A activation and beta 1 integrin function; examine whether these processes regulate collagen degradation.

Experimental Approaches: Filamin A-deficient fibroblasts transfected with wild-type filamin A or filamin A domain deletion mutants that fail to bind beta 1 integrin or prevent filamin A phosphorylation and activation will be plated on fibrillar collagen and treated with IL-1. Filamin A activation will be assessed with phospho-antibodies; 1 integrin activation will be assessed with neo-epitope antibodies. Collagen phagocytosis and proteolysis will be assessed with collagen internalization and fragmentation assays, and by measurement of collagen-degrading enzymes.

Significance: An improved understanding of the molecular mechanisms by which filamin A controls matrix remodelling may suggest novel treatment strategies for clinical management of connective tissue degradation in inflammatory diseases such as arthritis and periodontitis.

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