

List of Supervisors and Projects for Summer Research Program 2012

1. Dr. Limor Avivi-Arber:

Project Title: Functional mapping of the tongue extrinsic muscles

Project description:

Background: The tongue plays a vital role in mastication, swallowing, articulation and breathing. It is composed of eight intrinsic and extrinsic muscles each contributing to tongue movement in different direction. The sensorimotor cortex plays a role in the control of tongue movements. However, there is limited research on the cortical control of individual tongue muscles. We use electrical stimulation of the sensorimotor cortex and recording of evoked electromyographic (EMG) activity to map the spatial-temporal organization of the motor representation of the different tongue muscles within the rat brain.

Hypothesis: Each of the different tongue muscles have a distinct yet overlapping motor representations within the sensorimotor cortex. Such spatial organization of the tongue muscles may be necessary for coordinating the vital motor functions of the tongue muscles.

Objective: To use electrical stimulation of the sensorimotor cortex and recording of evoked EMG activity to map the spatial-temporal organization of the tongue muscles within the cytoarchitecturally defined M1.

Experimental Plan: Ten adult male Sprague-Dawley rats underwent systematic mapping of the left M1. EMG electrodes were inserted into the hyoglossus, styloglossus and genioglossus muscles. Cortical sites from which electrical stimulation evoked EMG activity in any of these muscles were noted. A modified Sihler's staining technique, that renders muscle tissues translucent, will be used to confirm the location of EMG electrodes.

Summer Student Project: The successful applicant will conduct histological staining and subsequent histological analysis on the tongue specimens to determine the location of the EMG electrodes and to correlate these results with the brain mapping results to determine the motor representation of each of the different tongue muscles. This study is an important step towards better understanding of the mechanisms underlying central control of tongue muscles and for the development of improved therapeutic approaches particularly in subjects suffering from tongue motor deficits.

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2. Dr. Grace de Souza:

Project Title: Validation of an artificial aging method for dental zirconia.

Project description:

Objectives: The aim of this study is to validate an artificial aging method for dental zirconia. Hypothesis: Dental and orthopedic zirconia present the same phase transformation process under artificial aging; there is a correlation between aging time and the amount of monoclinic phase; there is an inverse correlation between monoclinic

phase and flexural strength. Rationale: Orthopedic yttria-partially stabilized tetragonal zirconia (3Y-TZP) presents phase transformation at body temperature, which compromises the material's mechanical properties and its clinical applications. An artificial aging method has been validated for orthopedic zirconia and it was observed that the amount of monoclinic phase increases with aging time according to the MAJ (Mehl–Avrami–Johnson) equation. Despite that, Y-TZP has been extensively employed as a core material in dental prostheses and an artificial aging method has not yet been validated. It is paramount to demonstrate and validate an artificial aging method for dental zirconia to safely predict the behavior of the material in the mouth. Experimental plan: One orthopedic (3Y-TZP, grain size of 0.2 μ m, Kyocera – Japan) and one dental (3Y-TZP, grain size of 0.5 μ m, Lava, 3M - US) zirconia will be used. Blocks will be cut (3mm thick) in a pre-sintered stage, both sides will be polished up to 1 μ m diamond paste and samples will be fully sintered. Specimens will be aged in the same autoclave unit (Tuttnauer tabletop) under 2 bars pressure and 134°C for: 0, 30, 60, 90, and 120 minutes. The amount of monoclinic phase (X-ray diffraction analysis) will be evaluated in half of the specimens (n=5) and its correlation with aging time will be considered to validate the MAJ equation for dental zirconia. Results will be compared between dental and orthopedic zirconia (Two-way ANOVA and Tukey Test). The other half of the specimens (n=5) will be used to evaluate the bi-axial flexural strength (piston on three-balls) to correlate the amount of monoclinic phase and flexural strength by two-way ANOVA and Tukey Test. Statistical significance will be preset ($\alpha=0.05$). Independent variables for both analyses will be material and aging period. The first dependent variable will be the amount of monoclinic phase and the second one will be flexural strength.

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3. Dr. Bernhard Ganss

Project 1 Title: Dynamic Light Scattering Studies to Analyze the Aggregation Behaviour of the novel enamel protein amelotin.

Project description:

Rationale: Amelotin (AMTN) is a recently discovered enamel protein that is predominantly expressed during the late stage of amelogenesis. AMTN, similar to the main structural enamel protein amelogenin (AMEL), forms aggregates in solution, which may reveal important clues about its biological function. Hypothesis: AMTN aggregation in solution is dependent on temperature, pH and calcium ion concentration. Objectives: a) to produce sufficient amounts of recombinant AMTN in bacteria and purify the protein via affinity chromatography; b) to determine the influence of temperature, pH and Ca ion concentration in dynamic light scattering (DLS) experiments. Experimental Plan: Recombinant human amelotin (rhAMTN) will be expressed in E. coli BL21-DE3 cells and purified via Ni-NTA affinity chromatography following an already established protocol. The N-terminal His6 tag will be removed by tryptic cleavage. The anticipated amount of rhAMTN required to conduct the studies proposed here is 3 mg. The protein will be dissolved in modified simulated body fluid (c-SBF) and the temperature-dependent kinetics of aggregate formation determined using a DLS instrument.

Aggregate size will be calculated from the values obtained. Similarly, the influence of pH (4.0 to 8.0 in a titration experiment) and Ca ion concentration (0.1 to 2.5 mM in a titration experiment) on rhAMTN aggregation will be determined. To determine the shape of aggregates formed they will be deposited on metal stubs and analyzed by SEM. Where possible, aggregates will also be analyzed by TEM to determine their ultrastructural organization.

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4. Dr. Bernhard Ganss

Project 2 Title: Assessing the role of AMTN in preserving the integrity of the dentogingival junction in a murine experimental periodontitis model.

Project description:

Rationale: Amelotin (AMTN) is a recently discovered enamel protein that is predominantly expressed during the late stage of amelogenesis during tooth eruption. After eruption its expression is restricted to junctional epithelial cells at the dentogingival junction. We have created AMTN-deficient mice that show overt signs of enamel hypomineralization and attrition in incisors. While the dentogingival junction appears unaltered, this could be due to the mice being housed in a specific pathogen-free (SPF) environment. In this project we would like to determine in a molar ligature model whether AMTN-deficient mice are more susceptible to dentogingival detachment than wild type animals. Hypothesis: AMTN-deficient mice show more ligature-induced dentogingival attachment loss than wild type animals. Objectives: to determine the importance of AMTN in maintaining dentogingival attachment. Experimental Plan: Wild type and AMTN-deficient mice will be anesthetized and a 9-0 silk suture will be placed into the gingival sulcus of the upper second molar and tied around the tooth using a surgeon's knot. One side will serve as control. The secure knot will be placed at the disto-palatal angle of the second maxillary molar and gently pushed under gingiva, into the sulcus. At the end of the procedure the ligature will sit passively in the gingival sulcus and be visible through the thin gingival margin. 5. Three weeks later, mice will be sacrificed by CO₂ asphyxiation, each maxilla cleaned and stained with methylene blue. Pictures of the upper jaw in x 25 magnification will be taken in axial plane and horizontal alveolar bone loss measured on the buccal side. The distance between the cemento-enamel junction (CEJ) and the alveolar bone crest (CEJ-ABC distance) will be determined as a measurement of alveolar bone loss. Histological paraffin sections in frontal plane through M2 including control and ligature side will be stained with H&E and Masson's trichrome for measurement of net connective tissue (CT) attachment loss (CEJ-coronal aspect of JE), CT width (apical aspect of JE-alveolar crest) and PDL width. Micro-CT measurements will be conducted to provide three-dimensional aspects of alveolar bone loss.

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5. Dr. Bernhard Ganss

Project 3 Title: Expression Profiling of two Novel Genes in the Enamel Gene Cluster.

Project description:

Rationale: The enamel gene cluster (EGC) on human chromosome 4 and mouse chromosome 5 contains genes for important enamel proteins such as ameloblastin, enamelin, amelotin and ODAM. Two hitherto uncharacterized genes, C4orf7 and C4orf35, are also located in this cluster. The expression of C4orf7 and C4orf35 has not been determined. Hypothesis: C4orf7 and C4orf35 are novel genes that are expressed in ameloblasts during characteristic stages of enamel formation. Objectives: To determine the mRNA expression profile of C4orf7 and C4orf35. Experimental Plan: cDNA sequences of the murine C4orf7 and C4orf35 orthologues have already been cloned in suitable plasmids to generate digoxigenin-labeled sense and antisense cRNA probes specifically for those transcripts. These cRNA probes will be generated using a commercial kit (Boehringer Mannheim DIG labeling kit) and applied to sagittal paraffin sections of demineralized mouse craniofacial structures including maxillae and mandibles at various ages (embryonic day 16, postnatals days 1, 5, 10 and 25; already available in the lab). These ages represent different important developmental stages of tooth formation and will reveal crucial stages of amelogenesis. cRNA probes will be hybridized to those tissue sections using established protocols, where the antisense cRNA probe is expected to hybridize to C4orf7 and C4orf35 transcripts, while the probe in sense orientation will serve as negative control. Sections will be stained with a DIG antibody to reveal the precise cellular location of transcripts, and counterstained with haematoxylin and eosin (H&E) or methyl green to reveal general tissue structures. This project has the potential to identify two novel genes that may play an important role during enamel formation.

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6. Dr. Michael Glogauer:

Project Title: Role of Adseverin in Osteoclastogenesis.

Project description:

Background: The alveolar bone supports and provides attachment for teeth to the jaws and is an essential component of masticatory and oral function. As a result this bone is subjected to high amplitude mechanical forces from mastication and parafunction, and is also resorbed in inflammatory lesions arising from high prevalence oral infections such as periodontitis. Preservation and maintenance of alveolar bone is very dependent on tight, balanced coupling between bone formation and resorption. Currently we do not have a fundamental understanding of the critical regulatory processes by which alveolar bone is preserved in the face of inflammation and mechanical loading.

Feasibility: We have developed a mouse model to study preservation of alveolar bone homeostasis during inflammatory challenge and have discovered that the formation of osteoclasts by fusion of mononuclear precursor cells is a critical, rate-limiting step in

alveolar bone resorption. We found that the novel actin severing protein adseverin, is required for osteoclast formation but the mechanisms by which it controls the fusion of osteoclast precursors into functional osteoclasts are undefined.

Hypothesis: Adseverin is expressed by nascent osteoclasts to enable subcortical actin remodeling and membrane fusion, which ultimately regulate osteoclast formation.

Objectives: Determine the importance of adseverin in osteoclast formation in alveolar bone of mice that are subjected to experimental periodontitis. Use our extensively characterized mouse alveolar bone model and ligature-induced experimental periodontitis to define the kinetics of bone loss and the formation of multinucleated osteoclasts on the alveolar bone surface. Apply our recently developed adseverin null mouse to determine the importance of adseverin in the preservation of alveolar bone and formation of multinucleated osteoclasts in experimental periodontitis.

Significance: Our preliminary observations that adseverin is required for osteoclastogenesis provide novel insights into the potential importance of this protein and actin assembly as central components of bone homeostasis not only in alveolar bone but more globally throughout the human skeleton.

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7. Dr. Boris Hinz :

Project 1 Title: Proteolytic degradation of extracellular matrix proteins by myofibroblasts is controlled by the mechanical microenvironment.

Background: Fibrosis is characterized by the accumulation and excessive remodelling of a collagen-rich extracellular matrix (ECM). The stiffness of fibrotic tissues controls the ECM remodelling activity of fibroblastic cells. Only in the context of a stiff ECM, non-contractile fibroblasts develop contractile stress fibres and differentiate into myofibroblasts. Myofibroblast contraction is detrimental by causing dramatic tissue deformations in fibrosis. ECM remodelling and compaction additionally involves controlled ECM degradation, which is mediated by specialized enzymes, called matrix metalloproteinases (MMPs). Preliminary findings in the lab show that various MMPs are consistently up-regulated in myofibroblast cultures on soft substrates compared with stiff culture conditions. We hypothesize that the mechanical state of the ECM provides important clues that control the remodelling activity of myofibroblasts.

Aim: To evaluate MMP-mediated ECM degradation as a function of the mechanical microenvironment.

Strategy: We will culture primary rat lung myofibroblasts on collagen type I-coated silicone elastomer substrates with defined Young's modulus (elasticity in kilopascal, kPa), ranging from normal lung-soft (3-5 kPa) to fibrotic-stiff (50-100 kPa). We will collect medium supernatants (secreted MMPs), extract ECM proteins (matrix-associated MMPs) and cell extracts (cell-bound MMPs, mainly MT-1 MMP) after different times of culture as a function of substrates stiffness. First, we will identify the most dramatically upregulated MMP on the protein level using antibody arrays. After restricting the number

of targets, we will assess a) MMP gene transcription (RT PCR and qRT PCR), b) MMP protein expression, secretion and localization (Western blotting, immunofluorescence), and c) MMP activity (zymography and Western blotting). To control whether MMP production/activity is indeed primarily controlled by substrate stiffness or secondarily by the effect that substrate stiffness has on myofibroblast differentiation, we will repeat selected experiments with lung myofibroblasts at different differentiation stages (modulated by inhibition of the pro-fibrotic growth factor TGF β 1), grown on standard plastic culture dishes.

Methods: The student will learn and employ primary cell culture, fluorescence microscopy, antibody array usage, protein biochemistry and Western analysis, production and handling of soft culture substrates, zymography, RT-PCR and qRT-PCR.

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8. Dr. Boris Hinz :

Project 2 Title: Substrate stiffness controls the phagocytic activity of myofibroblasts.

Background: Fibrosis is characterized by the accumulation and excessive remodelling of a collagen-rich extracellular matrix (ECM). The stiffness of fibrotic tissues controls the ECM remodelling activity of fibroblastic cells. In the context of a stiff ECM, non-contractile fibroblasts differentiate into contractile myofibroblasts. Myofibroblast contraction is detrimental by causing dramatic tissue deformations in fibrosis. ECM remodelling not only involves ECM production, secretion and contraction but controlled breakdown. One possible mechanism for ECM breakdown is internalization of ECM by phagocytosis and subsequent lysosomal degradation. Preliminary findings in the lab suggest that ECM stiffness affects the phagocytic activity of myofibroblasts. A systematic analysis of this finding remains elusive.

Aim: To evaluate the phagocytic activity of myofibroblasts as a function of their mechanical microenvironment.

Strategy: We will culture primary rat lung myofibroblasts on silicone elastomer substrates with defined Young's modulus (elasticity in kiloPascal), ranging from normal lung-soft (3-5 kPa) to fibrotic-stiff (50-100 kPa). To quantify phagocytosis, we will seed fluorescent microparticles (diameter 1-10 μ m) onto myofibroblasts cultured under different mechanical conditions and for different times. Microbeads will be coated with collagen I and fibronectin. We will assess: a) particle attachment kinetics by analyzing the recruitment of focal adhesion proteins to membrane-bound beads using immunofluorescence and Western analysis of extracted beads; b) internalization kinetics by surface biotinylation and subsequent analysis of surface-bound (biotinylated) versus internalized (non-biotinylated) beads. To control whether phagocytosis is primarily controlled by substrate stiffness or secondarily by the effect that substrate stiffness has on myofibroblast differentiation, we will repeat selected experiments with lung

myofibroblasts at different differentiation stages (modulated by inhibition of the pro-fibrotic growth factor TGF β 1), grown on standard plastic culture dishes.

Methods: The student will learn and employ primary cell culture, fluorescence microscopy, confocal microscopy, protein biochemistry and Western analysis, production and handling of soft culture substrates, preparation of samples for mass spectroscopy.

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9. Dr. Boris Hinz :

Project 3 Title: Differential effects of the pro-fibrotic growth factor TGF β 1 on fibroblasts grown under different mechanical conditions.

Background: Fibroblastic cells differentiate into myofibroblasts by acquiring smooth muscle contractile features and heightened extracellular matrix (ECM) protein synthesizing activity in response to tissue injury, e.g., cardiac infarct, dermal abrasion, or trauma to the lung. These cells play a fundamental role in remodeling damaged tissues and in re-establishing mechanical integrity. Whereas controlled myofibroblast activity is beneficial for normal tissue repair it is detrimental when it becomes excessive and causes the tissue deformations characteristic for fibrosis. Fibroblast-to-myofibroblast differentiation is induced by transforming growth factor beta (TGF β 1) in the presence of mechanical stress, i.e. a stiff ECM. Only when both factors are present, myofibroblasts de novo express α -smooth muscle actin (α -SMA) and develop their highly contractile phenotype. However, it remains elusive if other pro-fibrotic actions of TGF β 1 are equally dependent on mechanical stress.

Aim: To discriminate mechano-dependent from mechano-independent pro-fibrotic actions of TGF β 1.

Strategy: Gene and transcript array analysis performed in the lab indicates that TGF β 1 differentially regulated pro-fibrotic gene expression in fibroblasts depending on the mechanical culture conditions. To follow-up this observation, we will culture primary dermal fibroblasts on silicone elastomer substrates with the defined Young's moduli (elasticity in kilopascal, kPa) of soft normal (4 kPa) and stiff fibrotic skin (60 kPa). Cells cultured in either mechanical condition will be treated with TGF β 1 over a 4 day culture period or left untreated. We will assess a variety of profibrotic characteristics of fibroblasts on the transcript and protein level: a) α -SMA expression and formation of stress fibres, b) expression of specific myofibroblast cell-cell-contact proteins, ECM protein production (collagen I, fibronectins, LTBP-1), c) matrix metalloproteinase production and secretion, growth factor production. Myofibroblast-unrelated genes (e.g., PAI-1) will be assessed as controls.

Methods: The student will learn and employ primary cell culture, fluorescence microscopy, confocal microscopy, protein biochemistry and Western analysis, production

and handling of soft culture substrates, RT-PCR and qRT-PCR, collagen and growth factor quantitative assays.

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10. Dr. Abdul Hakeem Mohamed Yakub :

Project Title: Finite Element (Dynamic) Analysis of Facial Growth using Burlington Growth study.

Though Cephalometric analysis of the face provides a guideline in diagnosis and treatment planning of patients in orthodontics, there are many pitfalls associated with validity of landmarks and errors in landmark identification. Above all, this static representation of growth gives very little information about the future dynamic changes of facial structures.

The aim of the study is to generate a finite element model of the face using the serial cephalometric radiographs from Burlington Growth Study. The study involves finite element modelling of different parts of facial skeleton using the finite element method for the same individual in different ages (time interval). The modelling would involve construction of areas/plots representing the facial and cranial bones which actively take part in the post natal growth namely cranial base, maxilla, mandible etc. If the relative displacements of these structures could be calculated in different ages then the predominant growth changes in these areas could be explained.

The study also could be extended to carry out the same for individuals with different facial types C1.I, C1.II and C1.III and comparing the differences in growth among these groups. Furthermore, if age related changes can be plotted as function of time for individuals belonging to same facial types, then it would be possible to construct a finite element model which may be used to predict the future growth changes.

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11. Dr. Tara Moriarty:

Project 1 Title: Development of intravital microscopy-based methods for investigation of spirochete contributions to periodontal and cardiovascular disease.

Project description: OBJECTIVE: Establish intravital microscopy-based approaches for direct visualization of periodontal disease progression and cardiovascular dissemination of oral microbes *Treponema denticola* and *Porphyromonas gingivalis*.

HYPOTHESIS: The development of intravital microscopy tools for studying periodontal disease progression and cardiovascular dissemination of oral microbes *T. denticola* and *P. gingivalis* will provide a powerful tool for investigating microbial behaviour and host responses in the natural habitat of living hosts.

RATIONALE: Spirochetes are important members of the oral microbial community, and are particularly prominent in periodontal disease. One powerful approach to studying the role of oral spirochetes in the etiology of periodontal and cardiovascular diseases is to directly visualize the behaviour of these bacteria in living hosts using intravital imaging. We have established a mouse model of *T. denticola*- and *P. gingivalis*-induced periodontal disease, are developing fluorescent strains of *T. denticola* which can be visualized in living hosts, and have obtained a fluorescent *P. gingivalis* strain from collaborators. Therefore, we have most of the tools in place to finally begin developing intravital microscopy methods for visualizing host-microbe interactions during progression of periodontal disease

EXPERIMENTAL PLAN: Determine if fluorescent *T. denticola* and *P. gingivalis* express sufficient levels of stable fluorescence over the course of longterm infections (5 weeks) to be visualized in the tissues of living mice by intravital microscopy. If expression plasmids are not stably maintained in the absence of antibiotic selection (after introduction into mice), we will stably integrate fluorophore expression cassettes into the chromosomes of *T. denticola* and *P. gingivalis*, using genetic methods with which I have extensive previous experience. If the summer student is successful in establishing protocols for visualizing fluorescent oral microbes during gingivitis and periodontal disease progression, she or he will begin developing methods for visualizing vascular structures and immune cells in the oral cavity, and studying entry of fluorescent oral microbes into the bloodstream of mice, by intravital microscopy.

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12. Dr. Tara Moriarty:

Project 2 Title: Investigation of endothelial receptors mediating vascular adhesion of the disseminating Lyme Disease Pathogen.

OBJECTIVE: Identify endothelial receptors mediating vascular adhesion of disseminating *Borrelia burgdorferi* (Bb).

HYPOTHESIS: The alternative fibronectin receptor CD44 mediates Bb vascular adhesion during dissemination.

BACKGROUND AND RATIONALE: Lyme arthritis is the most common clinical complication of Lyme disease, a persistent infection caused by the spirochete *Borrelia burgdorferi* (Bb). Bb disseminate to target sites such as joints via the vasculature. A key step in dissemination into target tissues is shear force-resistant adhesion of Bb to luminal vascular surfaces, which permits circulating bacteria to decelerate sufficiently for migration along endothelial surfaces to occur. Initiation of vascular adhesion in joints and skin is mediated by plasma fibronectin (Fn) and the Fn-binding Bb protein BBK32, The endothelial Fn receptor(s) targeted during the initiation steps of Bb vascular interactions

have not yet been identified. Candidate adhesion receptors include integrins $\alpha 4\beta 1$ and $\alpha 4\beta 7$, and CD44.

EXPERIMENTAL PLAN: The summer student will determine which candidate Fn receptors are appropriately expressed and localized at Bb adhesion sites in confluent endothelial monolayers in vitro, and in the vasculature of uninfected and infected mice in vivo by in situ immunofluorescence microscopy. If time permits, she or he will investigate the contributions of appropriately expressed and localized receptors to Bb vascular adhesion in an in vitro flow chamber model system, using commercially available antibody- and peptide-based reagents which functionally block receptor function. Most materials and techniques required to complete this project are already available in my lab or commercially. Ms Parikh will receive technical assistance and training in in situ immunofluorescence from collaborators.

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13. Dr. Tara Moriarty:

Project 4 Title: Role of TLR4 in enhancing host susceptibility to disseminated Lyme disease in response to diet-induced obesity.

OBJECTIVE: Determine if diet-induced obesity enhances host susceptibility to disseminated Lyme disease in a TLR4-dependent fashion.

HYPOTHESIS: TLR4 is required for enhanced host susceptibility to disseminated Lyme disease in response to high fat diet.

BACKGROUND AND RATIONALE: Lyme disease is the most prevalent vector-borne infection in the industrialized world, and its incidence is increasing rapidly, in parallel with increasing rates of obesity. Lyme disease is caused by the bacterium *Borrelia burgdorferi* (Bb). We recently found that high fat diet-induced obesity greatly enhances susceptibility of mice to disseminated Bb infection, suggesting that increasing obesity rates may contribute to the rising incidence of Lyme disease. Diet-induced obesity is dependent on toll-like receptor 4 (TLR4). Measurement of disseminated Bb infection in mice deficient for TLR4 will allow us to determine if enhanced susceptibility of hosts fed high fat diet is attributable to TLR4 function. Two of the most widely used mouse models of Lyme disease are mouse strains C3H/HeJ and C3H/HeN, which differ in the expression of TLR4. Both strains are equally susceptible to Bb infection under normal dietary conditions, but it is unknown if obesity-inducing high fat diet alters levels of disseminated infection and resulting Lyme disease pathologies in these animals.

EXPERIMENTAL PLAN: We will precondition C3H/HeJ and C3H/HeN mice with normal and obesity-inducing high fat chow for 5 weeks before infection with Bb. Three weeks after infection, the summer student will measure the effect of diet-induced obesity and TLR4 status on disseminated infection by qPCR measurement of bacterial burden in multiple tissues affected by Lyme disease (heart, joints, bladder, liver, lung, brain, skin,

ear). We will quantitatively score inflammatory Lyme disease pathologies in joints and heart using standard histological methods, and will measure levels of proinflammatory cytokines in all tissues by qRT-PCR and ELISA. Preconditioning will be performed before the beginning of the student's summer project to ensure timely completion of data analysis.

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14. Dr. Tara Moriarty:

Project 5 Title: Role of diabetes and atherosclerosis in enhancing host susceptibility to disseminated Lyme disease.

OBJECTIVE: Determine if host susceptibility to disseminated Lyme disease is enhanced in mouse models of atherosclerosis and diabetes.

HYPOTHESIS: Atherosclerosis and diabetes render hosts more susceptible to disseminated Lyme disease.

BACKGROUND AND RATIONALE: Lyme disease is the most prevalent vector-borne infection in the industrialized world, and its incidence is increasing rapidly, in parallel with increasing rates of obesity. Lyme disease is caused by the bacterium *Borrelia burgdorferi* (Bb). We recently found that high fat diet-induced obesity greatly enhances susceptibility of mice to disseminated Bb infection, suggesting that increasing obesity rates may contribute to the rising incidence of Lyme disease. Diabetes and atherosclerosis are common features of diet-induced obesity in humans. Mice fed obesity-inducing high fat diet exhibit elevated blood glucose and increased prevalence of fatty streaks in blood vessels susceptible to atherosclerosis. Enhanced susceptibility of hosts to disseminated Bb infection in response to diet-induced obesity could be dependent on atherosclerotic or diabetic pathologies, or both. We will determine if disseminated Bb infection is enhanced in mouse models of atherosclerosis and diabetes.

EXPERIMENTAL PLAN: We will precondition C3H/HeN mice with atherogenic chow for 10 weeks before infection with Bb. Hyperglycemia will be induced in mice before infection by streptozotocin treatment. Three weeks after infection, the summer student will measure the effect of atherogenic and hyperglycemia-inducing treatments on disseminated infection by qPCR measurement of bacterial burden in multiple tissues affected by Lyme disease (heart, joints, bladder, liver, lung, brain, skin, ear). We will quantitatively score inflammatory Lyme disease pathologies in joints and heart using standard histological methods, and will measure levels of proinflammatory cytokines in all tissues by qRT-PCR and ELISA. Dietary conditioning and streptozotocin treatment will be performed before the beginning of the student's summer project to ensure timely completion of data analysis.

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15. Dr. Paul Santerre:

Project 1 Title: Biodegradation study of a novel antimicrobial polymer with potential applications in dental composites.

Project description: Composite resins experience biological breakdown in the oral cavity that results in secondary caries and marginal breakdown, accounting for approximately 60% of all restoration replacements.¹ An innovative solution to the current problem of secondary caries is an antimicrobial composite with drugs chemically bound within the resin matrix.

Objective: The objective of this project will be to study the biodegradation of drug polymers developed from the use of novel antimicrobial monomers, using simulated human saliva esterases (SHSE).

Hypothesis: By covalently binding drugs into the backbone of biodegradable urethane drug vinyl (UDV) polymer as main chain monomers during synthesis, antibiotic free form drugs will only be released once salivary enzyme trigger the degradation of the polymer. It is hypothesized that the novel polymer will be hydrolyzed by cholesterol esterase (CE) activity to yield pure drug (Ciprofloxacin).

Methods: Cylindrical pellets (6 x 2 mm) of the drug polymer will be prepared by light curing in a Teflon mold. The drug polymer will be incubated in phosphate buffer and CE solution (pH 7.0 at 37°C) and analyzed for degradation products using High Performance Liquid Chromatography (HPLC), UV Spectrometry, Nuclear Magnetic Resonance and Mass spectrometry (MS). The solutions, buffer and CE complex, will be replenished frequently in order to maintain enzymatic activity. The biodegradation characteristics will be studied weekly for a period of 4 weeks (n=3). Initially a small aliquot will be removed for a time zero sample point and all removed incubated solutions will accumulate over a period of 7 days, pooled and then analyzed. At the end of the degradation periods, acetonitrile or methanol will be added to denature the enzymes and cease activity and all samples will be frozen in liquid nitrogen and stored at -80 °C until analysis. The HPLC fractions containing individual degradation products will be collected based on the start and end of the product peaks in the HPLC chromatograms. To determine the chemical composition, the extracted products will be studied using MS.

Reference: [1] Li F *et. al.* J Dent Res 2009; 88(4): 372-376.

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16. Dr. Paul Santerre:

Project 2 Title: Development of a novel antimicrobial monomer with potential applications in

Project description: An antimicrobial composite with drugs chemically bound is an innovative solution for the current problem of secondary caries and marginal breakdown, which is responsible for approximately 60% of all restoration replacements.¹ Such composite will improve the clinical function of restorations that will have both physical and economical impact in the clinical practice of dentistry.

Objective: The objective of this project will be to synthesize a monomer incorporating antibiotics (specifically Metronidazole (MN)) into the resin component of a restorative material. A synthesis methodology will be established and the monomer will be studied for their potential to be polymerized.

Hypothesis: It is hypothesized that a Metronidazole polymerizable monomer can be formed for incorporating into the resin component of restorative composites.

Methods: MN contains one hydroxyl group that can be coupled with the carboxylic group on methacrylate acid (MAA) or the isocyanate group (NCO) of lysine diisocyanate (LDI) in pendent mode. Thereby providing a vinyl group that would allow the drug to be incorporated into a chain growth polymerized network. Proton Nuclear Magnetic Resonance, Mass Spectroscopy and Infrared Spectroscopy will be used to characterize the synthesized monomers.

The monomer will be synthesized by coupling MN to LDI in pendent mode. The reaction will be carried out with a stoichiometry of 2:1 of MN: LDI, respectively. Subsequently, the ester group on the LDI moiety can be hydrolyzed and converted to a carboxylic acid functional group following previously established methodology using enzymes to hydrolyze the ester bond.² After hydrolysis, it will react with hydroxyethylmethacrylate (HEMA) in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and 4-dimethylamino pyridine (EDC/DMAP) as catalyst. This reaction will be carried out with a stoichiometry of 2:1:1 of MN: LDI: HEMA, respectively.

As an alternative, MN can also be coupled with methacrylate acid (MAA) thereby providing a vinyl group that would allow the drug to be incorporated into a chain growth polymerized network. Coupling of MN with LDI may provide better mechanical properties because of the longer chain length and more branched structure of the pendant chain. Therefore, coupling to LDI can be more beneficial if hydrolyzation of the methyl ester of the LDI does not affect the urethane bonds formed between the NCO on LDI and the OH on MN.

Reference: [1] Li F *et. al.* J Dent Res 2009; 88(4): 372-376. [2] Yamamoto N *et. al.* Reactive and Functional Polymers 2007; 67 (11): 1338-1345.

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17. Dr. Paul Santerre:

Project 3 Title: Myofibroblast activation and deactivation of human gingival fibroblasts cultured on degradable/polar/hydrophobic/ionic polyurethanes under media perfusion.

Project description: Rationale: Myofibroblasts are important players in wound healing; they are highly contractile cells that produce collagen, which helps in wound closure and extracellular matrix (ECM) remodeling [1]. Periodontal diseases cause breakdown in the tooth-supporting tissue that subsequently leads to bone resorption [2]. Tissue engineering is a promising way to regenerate gingival tissue and previous studies showed that culturing human gingival fibroblasts (HGF) on degradable/polar/hydrophobic/ionic polyurethanes (D-PHI) with media perfusion promoted cell growth and collagen production. It is unclear, however, whether these cells have differentiated into myofibroblasts, which can be characterized by the cells' relatively high α -SMA and ED-A fibronectin (Fn) levels, stress fibre formation, and *de novo* collagen synthesis.

Objectives: The objectives of this study are: (i) to visualize α -SMA expression and stress fibre formation in HGF at various time points during culture period under media perfusion, (ii) to show the physiological relevance of α -SMA expression with respect to its importance in myofibroblast differentiation by measuring ED-A Fn expression, and (iii) to visualize collagen network produced by HGF. **Experimental Plan:** Visualization of α -SMA expression and stress fibres will be done with immunofluorescence staining and an antibody against ED-A Fn will be used to verify the presence of myofibroblasts. The collagen fibre network will be visualized using histology with picrosirius red staining. **Hypothesis:** It is hypothesized that HGF cultured on D-PHI under media perfusion undergo activation towards a myofibroblastic state early in the culture period (characterized by the upregulation of α -SMA and ED-A Fn as well as stress fibre formation). In the long-term culture, the activated myofibroblasts will deactivate (i.e. downregulation of α -SMA and ED-A Fn). **Significance:** The possible activation of myofibroblasts on D-PHI may be beneficial for wound healing since highly contractile cells with collagen production are desired to accelerate wound healing.

References: [1] Hinz B. J Biomechanics 2010; 43: 146-55. [2] Moharamzadeh K., *et al.* J. Dent. Res., 2007; 86(2): 115–24.

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Please note: PI Projects listed below have already been matched with a student.

18. Dr. Amir Azarpazhooh:

Project Title: A Critical Appraisal of the Current Quality of Clinical Evidence in Dentistry.

Project description: Integrating clinical expertise and patient values, Evidence-Based Dentistry (EBD) focuses to answer clinical questions using the best available evidence. As EBD gains recognition, the trend towards using its concepts as a means of improving healthcare guidelines has become common in the healthcare field. As the extent to which high quality evidence is reported within the various fields of dentistry remains uncertain, the objective of this proposed series of studies will be to evaluate the quality of clinical research in Dentistry and how it has changed over the last ten years, and to propose steps to suggest how it needs to improve.

The proposed studies will be the first of a series of studies evaluating the quality of clinical research within the various specializations of dentistry. The studies proposed for work during the summer of 2012 are as follows:

1. The Quality of Evidence in the Endodontics Literature: Has Quality Improved over the Past Decade?
2. The Quality of Evidence in the Periodontology Literature: Has Quality Improved over the Past Decade?
3. The Quality of Evidence in the Implantology Literature: Has Quality Improved over the Past Decade?

In addition to the aforementioned studies, a series of related studies focusing on the quality of the Randomized Controlled Trials within these fields will be conducted. For this summer, the focus will similarly be in Endodontics, Periodontology, and Implantology.

The primary objectives of these studies is to assess the quality of the evidence reported in the top journals of each of the aforementioned specialties, and analyze how it has changed over the last decade. Students will conduct a systematic review of the top three journals within each of the aforementioned specialties, critiquing all clinical studies published in the years 2000 and 2010, and statistically analyzing the data for trends in the quality of the published studies within the last decade.

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19. Dr. Siew-Ging Gong:

Project 1 Title: Gene Expression in the Developing Mid-Face

Project description: Development of the mid-face during embryogenesis is under tight spatial and temporal genetic regulation. At the molecular levels, not much is known of the players regulating the proper shape, size, and differentiation of the mid-face that give rise to specific facial structures. We have previously conducted a microarray analysis to identify genes with potential roles in mediating growth and patterning of the medial versus the lateral parts of the mid-face. Identification and functional characterization of region-specific mid-facial genes are critical to understanding the development and regulation of regional differences during normal and abnormal craniofacial development. The objective of this study, therefore, is to characterize the quantitative, spatial and temporal expression patterns of genes from the original microarray data in wild-type mouse embryos at critical stages of mid-facial development. To achieve our goals, real time quantitative RT-PCR with gene-specific primers will be used to quantitate gene expression levels between the lateral and medial parts of the mid-face. Spatial and temporal expression patterns of these genes will be characterized by performing in situ hybridization on specific parts of the mid-face in embryonic day 9.5-15.5 mouse embryos using whole mount embryos and/or sections of the craniofacial region.

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20. Dr. Siew-Ging Gong:

Project 2 Title: Identifying key protein-protein interactions of FLRT2 in mediating cell adhesion during mid-face development.

Project description: During early craniofacial development, cranial neural crest cells (CNCC) migrate ventrally, condense, and differentiate at specific sites to give rise to most of the skeletal elements and cartilages of the craniofacial region. We showed that a member of the Fibronectin Leucine Rich Transmembrane Protein, FLRT2, is highly expressed in the CNCC during migration and condensation preceding nasal septal chondrogenesis and plays a role in regulating cell-cell interactions and cell-migration in a chondroprogenitor cell line. In an attempt to identify proteins that interact with FLRT2 during craniofacial development, a mass spectrometric study was conducted using tissue lysates from the developing mid-facial region. A number of proteins involved in cell movement were identified in the screen. Our hypothesis is that these proteins interact with FLRT2 to regulate cell movement in the developing mid-facial region. The objectives of this study are to (1) verify the interactions of these proteins with FLRT2 by performing co-immunoprecipitation experiments with mid-facial tissue lysates, and (2) characterize the expression of the verified proteins in the developing mid-facial region by immunohistochemistry. Future plans include the use of the primary nasal septal chondrocyte cell culture to test the roles of FLRT2 and its interacting partners in regulating cell movement. Data generated from the proposed plans will increase our understanding of the basic cellular and molecular mechanisms of CNCC cell migration and differentiation during craniofacial growth.

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21. Dr. Ernie Lam:

Project Title: Radiation dose calculations for small field cone beam CT imaging of the temporomandibular joints.

Project description: Background: Computed tomography is the imaging modality of choice for depicting osseous abnormalities of the temporomandibular joint (TMJ). The Faculty's current technique for TMJ imaging involves the use of the Hitachi CB MercuRay (Tokyo, Japan) cone beam CT system operating with a 9" field-of-view and 0.29 mm voxel size at 100 kVp and 10 mA. We have determined the effective radiation dose for TMJ imaging using this system to be 223.6 ± 1.1 microSievert (uSv) (Lukat and Lam, unpublished). Recently, we have begun experimenting with TMJ imaging using the Faculty's small field-of-view cone beam CT system, the Kodak 9000 3D (Carestream Kodak, Rochester, NY). This system utilizes a 5 cm (diameter) by 3.7 cm field of view and 0.076 mm voxel size, and operates with 4 different kVp and mA pre-sets depending on patient size; 68kVp/6.3mA for children, 70kVp/8mA for adolescents/small adults, 70 kVp/10mA for medium/average adults and 74kVp/10mA for large adults. The doses for the average adult have been determined to be 9.7 ± 0.1 uSv and 21.3 ± 0.4 uSv for unilateral and bilateral TMJ examinations, respectively. Objectives: The purpose of the study will be to determine the dose reductions that can be achieved using the Kodak 9000 3D machine for TMJ imaging using the child and adolescent/small adult kVp and mA settings. Hypothesis: We hypothesize a 20% to 50% dose reduction when the child and adolescent/small adult techniques are used for TMJ imaging. Rationale: Reducing kVp and/or mA relative to the adult technique ensures that doses to children and adolescents, the most vulnerable in our population, will be as low as reasonably achievable. Experimental Plan: Twenty-five optically stimulated luminescence (OSL) dosimeters will be placed in various locations in an anthropomorphic RANDO® Man phantom to measure absorbed radiation doses for tissues of the head and neck using both the child and adolescent/small adult TMJ imaging techniques. Effective doses for the two imaging techniques will be calculated using the 2007 International Commission on Radiological Protection tissue weighting factor recommendations. Dosimetric measurements will be performed in triplicate for each technique, and effective doses calculated. Already, we have demonstrated in preliminary data (Lukat and Lam, unpublished) that a dose reduction of greater than 90% can be achieved when the small field system is chosen. We expect to see further substantive reductions from these experiments.

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22. Dr. Christopher McCulloch:

Project 1 Title: Role of Focal Adhesion Kinase in Heart Failure.

Project description: Rationale: The pressure or volume overloaded heart exhibits hypertrophic growth of the myocardium and transformation of cardiac fibroblasts into myofibroblasts. Cardiac myofibroblasts secrete fibrillar collagens into the interstitium that increase myocardial stiffness and promote ventricular diastolic dysfunction. Accordingly, cardiac myofibroblasts may be key mediators of hypertrophic responses to cardiac overload, including fibrosis and scarring. The aim is to understand how mechanical forces mediate the differentiation of cardiac myofibroblasts in cardiac hypertrophy.

Background: Cardiac fibroblasts respond to chronic overload by increased proliferation, elevated remodeling of matrix proteins and by a switch to a myofibroblastic phenotype in which cells express abundant alpha-smooth muscle actin (SMA). Expression of SMA increases myofibroblast contraction contributing to further stiffening of the scar. The mechanisms by which mechanical loading mediates myofibroblast differentiation are not defined. Our most recent data indicate that when tensile forces are applied to fibroblasts, the SMA promoter is activated through a signaling pathway that may involve the focal adhesion kinase (FAK) and the actin binding protein gelsolin. This pathway may mediate actin

filament assembly, which is required for nuclear translocation of the transcriptional coactivator

MRTF-A, activation of the SMA promoter, SMA expression and myofibroblast differentiation.

Research Question: How do mechanically-activated signals regulate SMA expression and myofibroblast differentiation?

Hypothesis: Force-induced SMA expression requires interactions between FAK and gelsolin to mediate actin filament-dependent activation of SMA promoter.

Objective: Assess how FAK at force-transfer sites interacts with gelsolin to enable activation of the SMA promoter.

Approach: Mouse cardiac fibroblasts will be subjected to well-defined, physiologically relevant forces applied by an intensively standardized system through collagen-coated magnetite beads attached to beta 1 integrins. In situ FAK recruitment, activation and association with gelsolin will be measured by phospho-specific antibodies, immunoprecipitation and with pull-down assays using purified proteins. In cells that are null for FAK, SMA promoter assays will be conducted to examine gene activation through this pathway.

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23. Dr. Christopher McCulloch:

Project 2 Title: Role of alpha 11 integrin in diabetic cardiomyopathy.

Project description: Background: Diabetic cardiomyopathy is a poorly characterized, frequently fatal disorder of increasing prevalence that involves disruption of the cardiac vasculature, disturbances of electrical conductivity and conversion of the normal cardiac interstitium into a disorganized and fibrotic matrix. This abnormal matrix disturbs the structural organization and electrical conductivity of myocytes, increases myocardial stiffness and promotes ventricular diastolic dysfunction. Currently the mechanism by which reactive glucose metabolites in diabetes mediate interstitial fibrosis is not defined. Preliminary data indicate that collagen glycation and alpha 11 integrin-mediated transformation of cell adhesion and function are critical for the interstitial fibrosis that occurs in diabetic cardiomyopathy.

Research Questions: How does diabetic glycation of collagen promote the pro-fibrotic phenotype, disrupt cardiac fibroblast matrix adhesions and enhance interstitial fibrosis?

Hypothesis: Alpha11 integrin and TGF-beta2 cooperatively enable the formation of pro-fibrotic cells that produce a fibrotic interstitium, which disrupts normal cardiac function in diabetic cardiomyopathy.

Objective: Examine the interacting roles of integrins and the TGF-beta signaling pathway that lead to pro-fibrotic cell conversion as a result of collagen glycation.

Approaches: Human or mouse cardiac fibroblasts will be cultured on vehicle or glycated collagen to model diabetes-induced alterations of the extracellular matrix. TGF-beta isoforms will be measured by ELISA and qRT-PCR. Activation of downstream targets of TGF-beta signaling (Smads and phospho-Smads) will be assessed by immunoblotting. Cell surface integrins will be measured by surface labeling, flow cytometry and immunoblotting.

Significance: An improved understanding of how the interstitium regulates the differentiation of cardiac myofibroblasts in diabetic cardiomyopathy may lead to the development of novel approaches for prevention of heart failure.

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24. Dr. Tara Moriarty:

Project 3 Title: Coordination of adhesion and motility in the presence of fluid shear forces by the Lyme Disease Pathogen.

Project description: OBJECTIVE: Use live cell imaging approaches to study the coordination of vascular adhesion and cellular motility by the disseminating Lyme disease pathogen, and the effect of fluid shear forces on these functions.

HYPOTHESIS: Coordination of adhesion and motility in migrating *Borrelia* is dependent on the ability of extracellular bacterial adhesion complexes to diffuse freely in a lateral direction through the bacterial outer membrane.

RATIONALE: Lyme arthritis is the most common clinical complication of Lyme disease, a persistent infection caused by the spirochete *Borrelia burgdorferi* (Bb). Bb disseminate to target sites such as joints via the vasculature. During dissemination, Bb crawl along the luminal surfaces of blood vessels to intercellular junctions through which they migrate to extravascular tissues in the joint. This capacity for selective localization to extravasation sites is likely critical for the ability to disseminate into target tissues, and could be an important target for the rational design of dissemination inhibitors. Crawling requires the coordinated ability to adhere to and move along the surface of substrates, and occurs in the context of significant fluid shear forces in flowing blood relative to the size of Bb bacteria. It is unknown how Bb coordinate adhesion and motility functions to enable migration to and through endothelial junctions during dissemination. The most likely explanation for the coordination of these seemingly contradictory functions is that adhesion complexes formed at bacterial surfaces are actually mobile, and diffuse rapidly from one end of the bacterium to the other in the fluid outer membrane. This hypothesis is easy to test, by studying the interaction of motile and non-motile bacteria with beads in fixed positions which are coated with antibodies to bacterial adhesion complexes.

EXPERIMENTAL PLAN: We will use high-speed videomicroscopy and latex beads coated with antibodies to the Bb vascular adhesion protein BBK32 (antibodies to be characterized in the course of the summer project) to investigate the movement of BBK32 adhesion complexes in the membranes of motile and non-motile Bb strains. All bacterial strains, reagents and high-speed live cell imaging methods required for this project are already established in the lab.

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25. Dr. Anuradha Prakki:

Project Title: Bond strength of repaired experimental resins incorporated with chlorhexidine or catechins.

Project description: Objective: To evaluate the effect of different drug ratios on the repair microtensile bond strength of experimental bis-GMA/TEGDMA resins incorporated with either chlorhexidine (CHX) or catechin (C). The Null Hypothesis is that different drug type and ratio has no effect upon bond strength when repairing aged resins. Rationale: In order to prevent caries progression, chlorhexidine and green tea polyphenols (catechins) that have antibacterial and matrix metalloproteinase inhibitory effects have been incorporated into resins. It has been reported that at some ratios these compounds can impair resins polymerization conversion by mopping up free radicals from the monomers via protonation. The availability of unreacted methacrylate groups on the resin surface is however necessary to allow chemical bonding with a new layer of resin and improve the repair strength. It is still unknown to whether these new materials can promote an adequate repair bond strength and if so what best drug ratio should be incorporated into monomers. Experimental Plan: Bis-GMA/TEGDMA comonomer (70/30 mol %) blocks (6mm x 6mm x 4mm) will be prepared, polished, aged (boiling in water for 8h), and assigned (n=4) to the following groups: (CHX) drug incorporated at 0,

0.2, 1.0 and 2.0 wt%; and (C) drug incorporated at 0, 0.2, 1.0 and 2.0 wt%. The work surface of each block will be treated with 37% phosphoric acid + adhesive resin (Single Bond 3M ESPE), and each group/block will be incrementally restored with a 4 mm resin layer incorporated with the same drug in all four ratios (0, 0.2, 1.0, and 2.0%). Each resin increment (2mm) will be polymerized for 40s. The specimens will be sectioned in two axes (x and y) with a diamond disk under cooling water in order to obtain bar (8mm x 1mm x 1mm) specimens of 1mm² of bonding area. The microtensile bond test will be accomplished at crosshead speed of 0.5mm-1. After tensile tests, the bonding interface will be evaluated for fracture mode in an optical microscope. Bond strength data (MPa) will be submitted to normality and homogeneity tests, after which the appropriate statistical analysis will be performed ($\alpha=0.05$).

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26. Dr. Craig Simmons:

Project Title: The role of C-type natriuretic peptide in aortic valve disease.

Project description: Aortic valve disease (AVD) is a cell-mediated pathology marked by pathological differentiation of valvular interstitial cells (VICs) and unchecked remodeling of the extracellular matrix (ECM). We have shown that C-type natriuretic peptide (CNP) inhibits these processes in vitro, suggesting it may maintain valve homeostasis in vivo. Objective: To determine the functional role of CNP in AVD in vivo. To do so, we will compare the extent of diet-induced valve disease in wild-type (WT) mice and in mice with a defective CNP receptor (Npr2).

Hypothesis: Based on the protective effect of CNP in vitro, we hypothesize that mice with defective CNP signaling will get more advanced valve disease more rapidly than WT mice. Approach: To test this hypothesis, we are using a series of assays, including ultrasound biomicroscopy, blood analysis, and (immuno)histochemical (IHC) staining. The focus of the proposed project will be the IHC. The experimental plan is as follows: After four months of high fat/high carbohydrate diet treatment, mice will be sacrificed, their hearts and aortic arches collected, formalin fixed, and paraffin-embedded (the blocks will be available at the beginning of the summer research program). Five micron sections will be cut and (immuno)stained for ECM (Movat's pentachrome), CNP, TGF-beta (associated with valve disease and antagonized by CNP in vitro), alpha-smooth muscle actin (for myofibroblasts), Runx2 (for osteoblasts), and Sox9 (for chondrocytes); myofibroblasts, osteoblasts, and chondrocytes are all observed in AVD. Histomorphometric analyses will be performed to quantify ECM composition (collagen, proteoglycans, elastin), extracellular CNP and TGFb expression (relative area), and cell phenotypes (proportion of marker-positive cells).

Significance: The significance of these studies is that they are expected to define the functional role of CNP in valve homeostasis and disease, and may support therapeutic strategies involving CNP.

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