

List of PI and Projects for Summer Student Program 2009

1. Principal Investigator: Dr. Limor Avivi-Arber, Department of Prosthodontics and Dr. Barry J. Sessle, Department of Physiology

Contact information: **Limor Avivi-Arber**, Tel. (416) 979-4900 ext. 4618, Email: limor.avivi.arber@utoronto.ca

Face motor cortex neuroplasticity associated with molar extraction and placement of dental implants in rats

The primary motor cortex of mammals has an organized representation of the body musculature. Our studies concern the primary motor cortex that control the orofacial musculature (i.e. face M1) and its capacity to undergo neuroplastic changes in association with various intraoral manipulations in rats, monkeys and in humans (for review, see Sessle et al 2007). This has clinical significance since such cortical changes may reflect or allow for functional adaptation (or maladaptation) of the masticatory system to the altered oral state or altered oral motor behaviour and may contribute to the mechanisms whereby patients undergoing oral rehabilitation can (or cannot) restore the lost orofacial sensorimotor functions. It is hoped that by understanding the neuroplastic capabilities of the face-M1 we will be able to further improve therapeutic strategies for the restoration of oral functions and consequently improve quality of life of our patients.

The objectives of the present study are to use intracortical microstimulation (ICMS) and recordings of evoked muscle electromyographic (EMG) activity to systematically map the motor representations of the jaw and tongue muscles within the histologically defined face-M1 of anaesthetised adult male Sprague-Dawley rats, and to test if changes occur in these motor representations 1 week following unilateral extractions of the maxillary molars.

Rats will be divided into 3 study groups. Under local and general anaesthesia, the “extraction” group (n=10) will receive extraction of the maxillary right molars; the “sham extraction” group (n=10) will receive the same treatment as the extraction group but without actual tooth extraction; the “naïve” group (n=10) will receive no treatment. Systematic ICMS mapping will be carried out at ~7 days post-operatively, under ketamine general anaesthesia. Histological cross sections will confirm the location of “positive ICMS sites” for which ICMS can evoke EMG activity in jaw and/ or tongue muscles. Customized software written in Spike2 script (CED, Cambridge Electronic Design, Cambridge, UK) and LabView (National Instruments, Austin, TX, USA) will be used to analyze data files. For each of the jaw or tongue muscles, the mean number of positive ICMS sites reflects the muscle motor representation within the face-M1. These motor representations will be compared across study groups.

The summer student will have the opportunity to participate in some of these experiments, and help to extract and process the data.

2. Principal Investigator: Dr. Cameron Clokie and Dr. Sean Peel

The effect of prior treatment with bisphosphonates on Bone Morphogenetic Protein (BMP) Induced bone formation.

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Objective

To analyse the bone formed in mice pre-treated with different doses of Zoledronate.

Rationale

Bisphosphonate associated osteonecrosis of the jaw (bONJ), is characterized by exposure of bone in the mandible or maxilla for more than 8 weeks in patients who have taken bisphosphonates (BP) in the absence of radiation therapy. Management of bONJ is limited, and is primarily aimed at treating infection and preventing the condition from worsening.

BPs are used in the treatment of osteoporosis, Paget's disease of bone and also as an adjunct therapy in cancer treatment where skeletal sites are involved. It is known that BP inhibits osteoclast formation and resorptive capacity, while stimulating cell death. This results in a reduction in bone resorption. However, there is a gap in our understanding of how bisphosphonates may interfere with bone healing and thus play a role in the development of ONJ.

BMPs have been used clinically to promote bone regeneration and may be useful in the treatment of osteonecrotic lesions. However it is unclear as to whether the prior and continued exposure of the subject to bisphosphonates will alter BMP induced bone formation.

Experimental Plan

Mice were treated with the Zoledronate 4 weeks prior to and on the day of BMP implantation. After a further 4 weeks the BMP induced bone ossicles were excised and analyzed by microCT. Other tissues were also collected for histological analysis

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The successful applicant will conduct histological analysis on the recovered bone ossicles and evaluate the type (immature vs mature) and quantity of bone within the ossicles. The results will be correlated with previously obtained mCT results.

The liver kidney and spleen will also be examined for signs of bisphosphonate induced toxicity.

The results of this pilot study will provide important information which will assist us in determining the potential usefulness of treating bONJ patients with BMPs.

3. Principal Investigator: Dr. Dennis G. Cvitkovitch **Dental Research Institute, Faculty of Dentistry, University of Toronto**

Project 1:

Investigation of acquired resistance against bacteriophages in *Streptococcus mutans*

Background: Bacteriophages (phages) are viruses that attack and kill bacteria. Bacteria have evolved several mechanisms to avoid infection including short direct repetitive DNA sequences, called clustered regularly interspaced short palindromic repeats (CRISPRs), which are homologous to bacteriophage genes. CRISPRs together with their CRISPR associated genes (*cas* genes) are believed to be involved in resistance to bacteriophage infections. Analysis of the *Streptococcus mutans* UA159 genome revealed at least 10 genes comprised of CRISPRs and/ or *cas* genes that were hypothesized to confer phage resistance.

Our lab is interested in identifying genetic factors and underlying pathways that mediate bacteriophage immunity in *S. mutans*. To accomplish this, we have first assessed phage resistance in several *S. mutans* strains challenged with virulent phage M102 in an infection assay. Interestingly, all *S. mutans* strains were resistant to phage infection, except strain OMZ381 that showed sensitivity to phage infection resulting in cell death. Hence, we now seek an answer to the following question: What genetic factors in the other *S. mutans* strains facilitated resistance against M102.

Experiments:

1. To elucidate the involvement of 10 putative *cas* genes in cell immunity against phage infections. We will do this by constructing knock-out mutants in these genes and by challenging the mutants with virulent phage.
2. To identify genes important for immunity against phage, we will use DNA microarrays to examine the transcriptome of phage infected and non-infected *S. mutans*.

4. Principal Investigator: Dr. Dennis G. Cvitkovitch
Dental Research Institute, Faculty of Dentistry, University of Toronto

Project 2:

Transcriptome analysis and antimicrobial sensitivity of a PknB mutant of *Streptococcus mutans*

Background: PknB is signal receptor in *S. mutans* that is a eukaryotic-type serine/threonine protein kinase (STPK) that affects biofilm formation, genetic competence, and growth at low pH. PknB contains extracellular PASTA domains that are predicted to bind to the D-ala-D-ala dipeptide of unlinked peptidoglycan in the bacterial cell wall. Since PknB is located in the cell membrane of *S. mutans*, we hypothesized that this sensor protein can monitor or detect cell wall fragments in the environment released by dividing or lysed bacteria, and respond by changing its cell physiology.

To study the putative role of PknB as sensor of cell wall fragments, we aim to perform the following studies:

- 1) Extract cell walls from *S. mutans* cultures.
- 2) Study the growth of UA159 and the PknB mutant under different concentration of extracted cell wall.
- 3) Study gene expression of *pknB* and other genes believed to encode putative cell wall fragment sensors in UA159 and PknB mutant strains.

5. Principal Investigator: Dr. Dennis G. Cvitkovitch
Dental Research Institute, Faculty of Dentistry, University of Toronto

Project 3:

Involvement of the Trk potassium transporter system in the acid tolerance response of *Streptococcus mutans*

Rationale: *Streptococcus mutans* plays a major etiological role in the formation of dental caries via acid production and demineralization of the tooth enamel. Its ability to produce acid in the presence of dietary sugars is complimented by its remarkable capacity to thrive under acidic low pH, thus making its ability to tolerate acid an important virulence factor and a significant area of scientific intrigue. *S. mutans* has been shown to possess constitutive and acid-induced protective mechanisms that help it adapt, tolerate and survive at low pH that inhibits the growth of other bacteria in the plaque biofilm, allowing *S. mutans* to flourish and cause caries. Hence, an understanding of the molecular pathways that contributes to its ability to tolerate acid can be useful to the development of strategies prevent *S. mutans*'-mediated caries development.

Background: Using *S. mutans* cells exposed to low pH, we identified inducible genes encoding a putative potassium uptake system (designated Trk). Importantly, a similar TrkA protein in *E. coli*, has been identified as a peripheral membrane protein involved in the uptake of potassium into the cell. Potassium uptake has been linked to the maintenance of intracellular pH in number of bacteria. However, the importance of the Trk system for acid tolerance of *S. mutans* has not been investigated. Hence, we aim to investigate the involvement of the Trk operon in the acidogenicity and acid tolerance of *S. mutans*.

Experimental Plan:

1. To monitor potassium transport in the wild type and Trk knockout mutants in *S. mutans*.
2. To utilize Trk mutants to understand how potassium uptake may enhance survival or biofilm formation under acid stress.

**6. Principal Investigator: Dr. Dennis G. Cvitkovitch
Dental Research Institute, Faculty of Dentistry, University of Toronto**

Project 4:

Effects of competence stimulating peptide on gene expression and genetic transformation of *Streptococcus mutans*

Background: Genetic transformation enables bacteria to uptake and incorporate foreign DNA into their genome. Transformable bacteria can acquire novel properties that can enhance their overall fitness and survival. For example, antibiotic resistance is acquired via genetic transformation – a process that allows harmful bacteria to proliferate despite our attempts to eradicate them. *Streptococcus mutans* is a naturally transformable cariogenic bacterium that resides in dental plaque. In *S. mutans*, transformation is dependent on a signal peptide designated called the competence stimulating peptide (CSP) encoded by the *comC* gene and detected by neighbouring *S. mutans* cells via a membrane-bound sensor encoded by *comD*. We have discovered that CSP has many important functions in addition to facilitating transformation and this molecule is especially important for *S. mutans* adaptation to growth on (tooth) surfaces as a member of dental plaque. An important goal in our lab is to investigate the underlying molecular pathways controlled by CSP and to determine the pathways and adaptive processes controlled by this important signal molecule.

Experiments:

1. Study the ability to uptake and incorporate DNA in various *S. mutans* strains and varying environmental conditions. For example, we will examine transformation frequency in cells grown as biofilms and planktonic cells with varying CSP concentrations using *S. mutans* wild type and ComC mutant strains.
2. To investigate the genes and pathways controlled by CSP, we will perform DNA microarrays derived from various transformation-deficient mutants grown as a biofilm exposed to CSP.

7. Principal Investigator: Dr. Leslie Laing Gibbard, Assistant Professor, Discipline of Prosthodontics, Faculty of Dentistry, University of Toronto

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The Effectiveness of Ozonated Water on the Sanitation of Denture Acrylic

Candida albicans is one of several oral micro-organism species that accumulates on the intaglio/impression surface of removable dentures. Various methods have been proposed to clean dentures effectively, including mechanical and chemical ones, with the intention of preventing denture stomatitis. The objective of this study will be to determine which of these methods as well as exposure to ozonated water, is the most effective. It is hypothesized that the ozonated water will be the most inexpensive and effective technique in reducing the number of fungal organisms on denture acrylic. *C. albicans* will be cultured in yeast-mould broth. Experimental denture plates will be prepared using heat-cured acrylic resin. The surfaces will be left either smooth or be roughened; be lined with COE-Soft (denture reline material) or not. The plates will then be incubated with fungus solution for 120 minutes at 37°C, washed 3 times and stored in Petri dishes for 20 minutes at room temperature. Plates will then be subjected to i) ozonated water prepared by the Lotus system a) in the form of a spray or b) by total immersion, ii) a commercial denture cleanser (Polydent), iii) ultrasonic cleansing, or iii) distilled water. Antimicrobial activity will then be determined for each method to determine which is the most effective and inexpensive for denture cleansing.

8. Principal Investigator: Dr. Chris McCulloch

Role of diabetes in generation of cardiovascular fibrosis

Funding: Heart and Stroke Foundation

Abstract: Diabetes mellitus is a high prevalence metabolic disease that is strongly associated with cardiomyopathy and the development of interstitial myocardial fibrosis. As cardiac fibrosis can be mediated by myofibroblasts, we will test the hypothesis that collagen glycation converts human cardiac fibroblasts to myofibroblasts. Collagen glycation will be modeled by the glucose metabolite, methylglyoxal (MGO). Cells will be cultured on MGO-treated collagen and the activity of the α -smooth muscle actin promoter and the level of protein expression of α -smooth muscle actin, ED-A fibronectin and N-cadherin will be measured. Remodeling of floating and stress-relaxed collagen gels will be measured with control and MGO-treated collagen. We will measure cell migration with Transwell assays. β_1 integrin activation will be measured by neo-epitope antibodies. We hope to determine whether collagen glycation augments the formation and migration of myofibroblasts, which are critical processes in the development of cardiac fibrosis in diabetes.

9. Principal Investigator: Dr. George Sandor and Dr. Sean Peel

Evaluation of MRI for monitoring vascularization during bone healing

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Objective

To correlate MRI and histological evaluation of vascularization during bone repair.

Rationale

The development of an adequate blood supply in a bony defect is essential for bone regeneration. Currently studies on neoangiogenesis and vascularization of bone defects require the sacrifice of the animal and histological processing of the tissue to image the blood vessels which form in the defect. This methodology is labor intensive, is semi-quantitative and only provides information as to what is happening at a single time-point. Further it is unable to demonstrate whether flow is present within the vessels identified.

Magnetic Resonance imaging (MRI) has been used to non-invasively monitor angiogenesis and measure blood flow in soft tissues. We wish to determine the potential of using MRI to monitor vascularization and quantitate blood flow during bone healing by comparing the results of the MRI scans with those obtained using histology at specific time points.

Experimental Plan

Bilateral critical sized defects were created in the calvaria of New Zealand White rabbits. Defects were filled with an extracellular matrix, with or without VEGF (to promote angiogenesis). Over the course of healing MRI data was obtained to assess vascularization and quantitate blood flow in vivo. Animals were sacrificed at 6 weeks and 12 weeks for further analysis.

Summer Student Project

The successful applicant will conduct histomorphometric analysis of the repair tissue to determine the microvascular density (MVD) within the defects and compare their results using the current histologic “gold standard” with those previously obtained using MRI.

The amount of bone present in the defects will also be measured by microCT and histomorphometry to determine whether we can demonstrate any correlation between blood flow as determined by MRI and bone repair.

This is a key and novel study in bone regeneration and will be the first to describe angiogenesis in bony defects using MRI in vivo. Moreover, the correlation and conclusions from this study will prove key for future investigations into angiogenesis and bone regeneration.

10. Principal Investigator: Dr. Ze'ev Seltzer

Project 1:

Human pain genetics

Contact info: Professor Ze'ev Seltzer, University of Toronto Centre for the Study of Pain; tel. (416)979-4900ext4433; fax (416)979-4936; email: zeev.seltzer@dentistry.utoronto.ca

Objective: Identifying genes that predispose carriers to developing neuropathic pain following nerve injury.

Hypotheses and Rationale: Polymorphisms in certain genes confer risk for neuropathic pain following nerve injury. Identifying such polymorphisms would enable pharmaceutical companies to develop better painkillers.

Experimental plan: The student will recruit participants for the study by using public transportation or his/her own car to meet with individuals that suffered spinal cord injury, hand/leg amputation, breast surgery to remove a tumour, or coronary artery bypass and grafting (CABG). These patients live in the GTA or come for treatments and follow up meetings with caregivers in the GTA. The student will interview the patients and fill out with them electronic pain phenotyping questionnaires on a laptop that will be given to them for the duration of the summer project. The student will send the filled questionnaires to the server of the study at the UofT and carry out statistical analyses of the data, and produce a summary report. Based on previous experience, ~5 patients can be interviewed per day, ~250 over the summer. It is possible that a similar project will take place in Cambodia and Vietnam, on limb amputees. The student(s) might be offered the opportunity of carrying out this field project over there. In this case, the study will additionally include a survey of neuropathic pain treatment needs and of rehabilitation needs of the surveyed population. All expenses will be covered.

This project will NOT contribute knowledge to any of the following areas: Arthritis, Cancer and cancer related research, Cardiovascular/heart disease, Diabetes/Insulin, Kidney, Respiratory-related diseases, Polio, Common cold.

11. Principal Investigator: Dr. Ze'ev Seltzer

Project 2:

Mouse pain genetics

Contact info: Professor Ze'ev Seltzer, University of Toronto Centre for the Study of Pain; tel. (416)979-4900ext4433; fax (416)979-4936; email: zeev.seltzer@dentistry.utoronto.ca

Objective: Identifying neuropathic pain genes in several neural structures of the mouse that process pain input (i.e., trigeminal and spinal dorsal root ganglia, trigeminal brainstem nuclear complex and spinal dorsal horn) following injury to a nerve innervating the face or leg.

Hypotheses and Rationale: Neuropathic pain is characterized by pain that spreads outside the field innervated by the injured nerve. This indicates that neuroplastic mechanisms took place in the CNS. Previous studies that were carried out in our lab, partly by summer students, showed that the extent of this spread is controlled genetically. The project offered to you aims at identifying the genes controlling this trait.

Experimental plan: The student will assist in ongoing studies of two graduate students in our group who phenotype male and female mice of 25 different strains. The student will

take part in sensory testing of intact, sham operated and infra-orbital nerve-injured mice and quantifying their responses to tactile and heat stimuli applied to select targets in the body.

This project will NOT contribute knowledge to any of the following areas: Arthritis, Cancer and cancer related research, Cardiovascular/heart disease, Diabetes/Insulin, Kidney, Respiratory-related diseases, Polio, Common cold.

12. Principal Investigator: Dr. Craig Simmons

Hemodynamic regulation of heart valve disease

Contact: Craig Simmons, e-mail: c.simmons@utoronto.ca, Tel: 416-946-0548, Fax: 416-978-7753

Research area: Cardiovascular/heart disease

Rationale: Sclerosis (thickening and hardening) of the aortic heart valve is a common and debilitating disease. Valve dysfunction in aortic sclerosis (AS) results from fibrotic changes caused by myofibroblasts, which differentiate from normal fibroblast-like valve interstitial cells (VICs). The factors that regulate myofibroblast differentiation in the valve are not well defined. We have shown that the endothelium in disease-protected regions of aortic valves expresses CNP, a soluble protein that may act in a paracrine manner to inhibit myofibroblast differentiation of VICs. CNP expression may be regulated by shear stress, as CNP is expressed by endothelium that experience high shear stress.

Objective: To determine if shear stress regulates CNP expression and endothelial regulation of myofibroblast differentiation.

Hypotheses: CNP is produced by valve endothelial cells (ECs) in response to high shear stress and acts in a paracrine manner to inhibit VIC myofibroblast differentiation.

Specific Aims:

- 1) To determine if shear stress regulates CNP expression by valve ECs.
- 2) To determine if shear stress-induced CNP expression by ECs regulates myofibroblast differentiation of VICs.

Experimental Plan: The student will use in vitro flow chambers to subject valve ECs to various levels of shear stress that mimic those experienced by the valve. Shear-dependent CNP expression will be measured by PCR, immunoblotting, and ELISA. VICs will be treated with conditioned media from ECs subjected to various levels of shear. Myofibroblast differentiation will be assessed by immunoblotting, immunostaining, and contraction assays. A causal link between EC-derived CNP and myofibroblast differentiation will be tested using a CNP receptor antagonist.