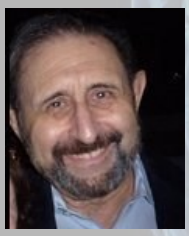
FIU | Biomedical Engineering

4th Annual Graduate Research Day

Friday, November 7, 2014

Featuring lectures by:



David E. Drew, Ph.D.
Joseph B. Platt Chair and Professor of Education
Claremont Graduate University
"How to Build a Successful Academic Career"

Joseph Culver, Ph.D.
Associate Professor
Washington University School of Medicine
"Optical Imaging of Distributed Brain Function and Networks"

Presented by:

Wallace H. Coulter Biomedical Engineering Distinguished Lecture Series
FIU Department of Biomedical Engineering

Florida International University, Engineering Center 2300
10555 W. Flagler Street, Miami, FL 33134
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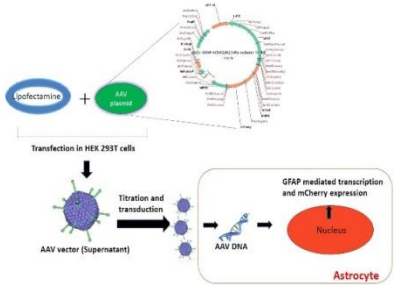
A quantitative evaluation of optogenetically-induced calcium signaling in astrocytes

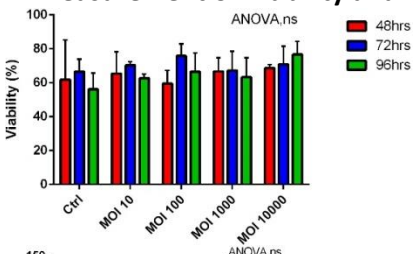
Authors: Lakshmini Balachandar[1], Andrea Raymond[2], Madhavan Nair[2], Jorge Riera Diaz[3]

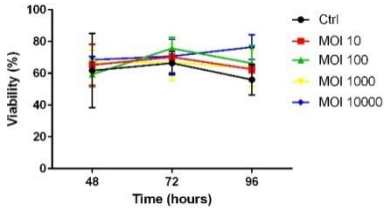
Major Advisor: Dr. Jorge Riera Diaz

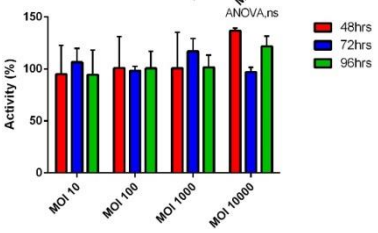
1. Materials and Methods

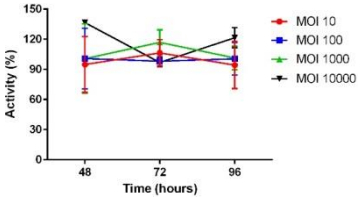


2. Measurement of Viability and Activity for different viral loads and transduction times

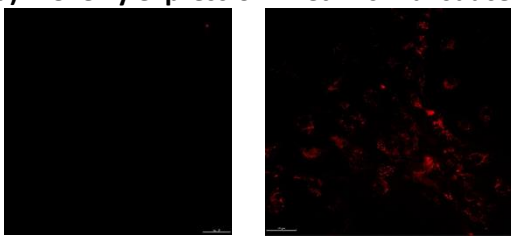




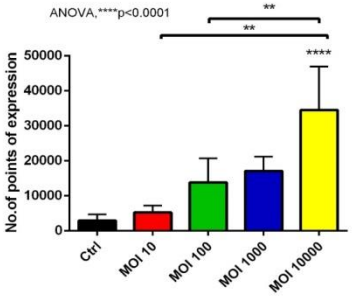




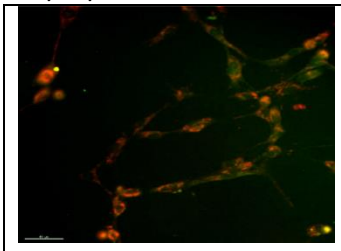
3(a) mCherry expression in Ctrl vs Transduced samples

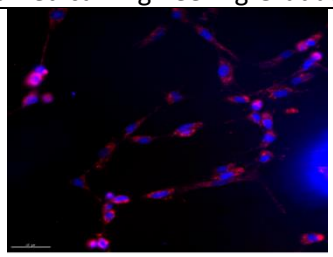


3(b) Plot of mCherry expression for varying viral loads

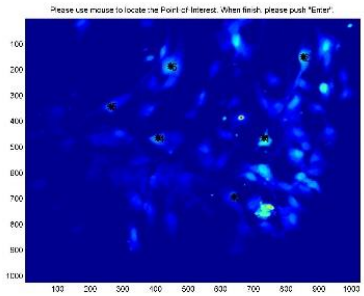


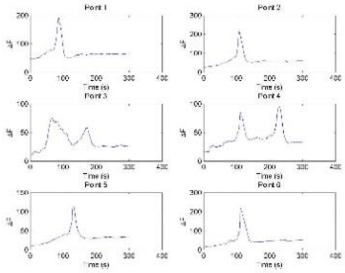
4.Calcium loading and morphometric analysis





5. Recording of Calcium activity enhanced by mCherry





Abstract

Optogenetics is a modern technique to understand functioning principles of targeted neurons and their circuits. Studies have shown opsin gene expression in neurons modulate neural activity by light stimulation, which has been expanded to non-neuronal cells - astrocytes (Figueiredo et al., 2010). However, the mechanisms by which light stimulation affects channelrhodopsins on astrocytes have not been quantified. The main purpose of this study is to provide a protocol for viral transduction parameters optimal to produce calcium signaling in rat astrocytes.

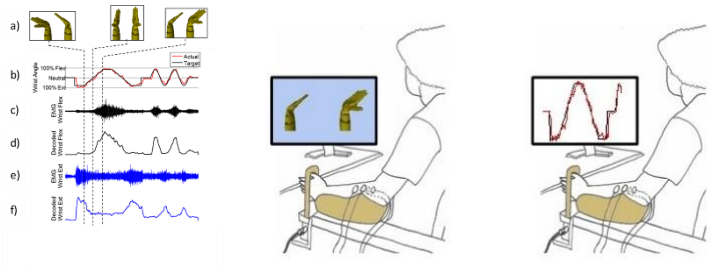
A DNA plasmid with an optogenetic probe (from Addgene) having a Chr2 and GFAP promoter was used to specifically target astrocytes. Creation of the virus by transformation, packaging by transfection and expression studies by transduction after titration were done. Primary rat astrocytes (Sciencell) were transduced with different viral loads at varying durations. Transfected astrocytes were stained with Sulphorhodamine 101 and Fluo-4 (Flourescent) to evaluate morphometric changes and light-induced calcium signaling measurement.

The astrocytes were transduced with increasing viral loads at various time points. The viability and activity of the cells (Fig. 2) indicate that there is no significant death/ reduction in activity of the astrocytes. However, significantly higher expression of mCherry is observed at higher viral loads (Fig. 3). Calcium signaling evoked optogenetically resembled those obtained with standard low-doses of exogenous glutamate (Fig. 5).
We developed a method to quantify and prove that the virus is suitable for a chronic study, and does not kill/ deteriorate the activity of rat astrocytes, while the expression of it increases significantly with viral load. In a second study, we will evaluate ideal stimulation parameters (e.g. intensity, duration, frequency) to evoke astrocytic calcium waves. This would thereby pave way to understand one of our long-term goals of the role of changes in calcium activity in astrocytes, as a result of epileptic seizures.

Does the nature of visual feedback provided when performing wrist flexion and extension movements impact performance?

Authors: iian Black, Anil Thota, James Abbas, Ranu Jung

Major Adviser: Ranu Jung



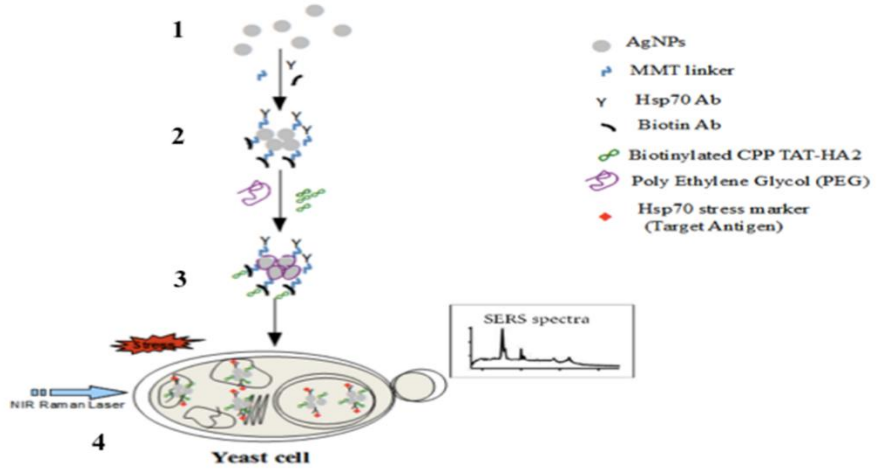
Abstract

It is not uncommon for upper-extremity amputees to discontinue use of their myoelectric prostheses, and studies have reported up to 75% rejection rates among this amputee group. Training regimens, both prior to receiving the prosthesis and afterwards, has shown to improve short term functional gains and long-term acceptance and incorporation of the device into activities of daily living. This work presents two training regimens requiring a healthy subject to activate wrist flexor and extensor muscles, muscles commonly used by amputees to control their prosthesis, and compares their impact on the subject's ability to precisely control muscle output to achieve simple target matching tasks.

Label-Free SERS Biosensor for Monitoring Environmental-stress

Authors: Vinay Bhardwaj, Supriya Srinivasan, Joshy F. John and Anthony J. McGoron

Major Adviser: Dr. Anthony J. McGoron



Abstract

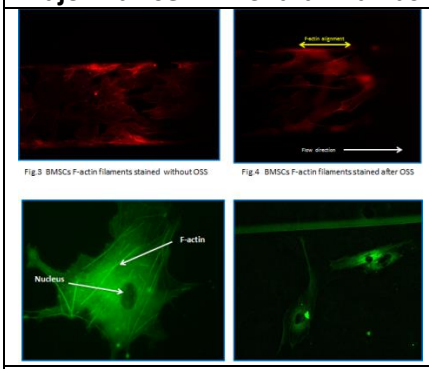
Increased threat of environmental contamination with toxins/stress might be deliberate (terrorism) or accidental; such as the sarin attack in Tokyo (Japan), *salmonella* attack in Oregon (USA), tragic factory accidents in Bhopal (India) and Chernobyl (formerly in Soviet Union). To avoid such attacks/accidents several "detect to protect" sensor devices have been developed and commercialized to date. But none of these existing biosensors meet the most demanding requirements; label free portable/wearable biosesnsor chip for real time monitoring of stress in whole cell/organism. Our group has proposed to fabricate an ultra-sensitive PCB-SIST chip, **P**ortable **C**ell-based **B**iosensor using **S**urface-enhanced Raman spectroscopy **I**mmune-**S**ensor **T**echnolog, using yeast as sensor organism, for environment-surveillance.

Silver nanoparticles (AgNPs)-based SERS immunosensor shows good correlation with standard ELISA technique, in detection of stress-marker protein, HSP70 in response to environmental-toxins (Environmental Protection Agency recommended Immediate Dangerous to Life and Health concentrations, EPA-IDLHs). The developed SIST Chip for environmental-surveillance meets the critical demand of **QuECHERS** (Quick, Easy, Cheap, Effective, Robust and Safe) **detect-to-protect** class of biosensor, terms coined by Department of Homeland Security. We developed a prototype SIST biosensor chip/kit for cell-free, end-point detection like ELISA. We look forward to develop a portable cell-based (PCB), dynamic sensor and compare with standard cytogenetic assays.

Bone Marrow Stem Cell Structural Reorganization after Flow Exposure: Relevance to the Valve Phenotype

Authors: G. Castellanos, L.Nassar, S. Rath, S. Ramaswamy

Major Adviser : Dr.Sharan Ramaswamy



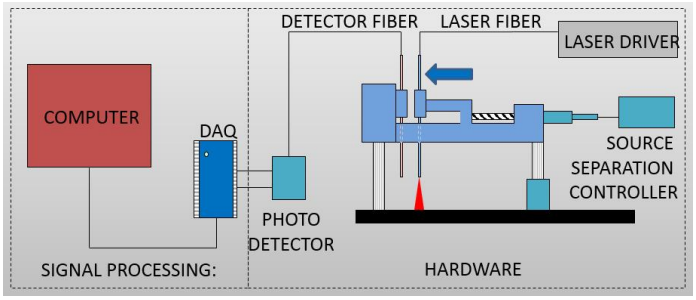
Abstract

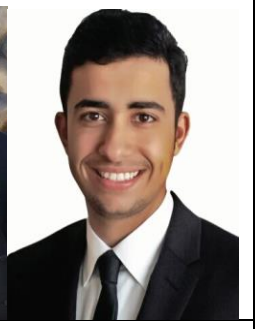
Tissue engineered heart valves (TEHV), based on bone marrow stem cells (BMSCs) and biodegradable scaffolds, have been investigated as the next step to current prosthesis limitations particularly lack of somatic growth, which is critical in the pediatric patient population[5]. BMSCs are a promising candidate cell source for tissue heart valve engineering due to its accessibility and phenotypic plasticity under biochemical and mechanical environments. Recent studies have shown that blood shear stress can regulate the proliferation and differentiation of MSCs through a variety of signaling pathways [6]. Heart valves experience mechanical stresses including cyclic flexure, tensile and oscillatory shear stress (OSS) during their lifetime [7]. We previously demonstrated that coupled flexure and flow environments augmented tissue formation using PGA:PLLA scaffolds seeded with BMSCs [8]. Changes in BMSCs' cytoskeleton have also been observed when BMSCs are exposed to OSS. Alterations in F-actin are closely linked to gene expression and protein synthesis in the mechanobiology of stem cells as well as in the differentiation of stem cells. However the pathway by which OSS affects the cell structural response, especially F-actin, of BMSCs is not well understood. Understanding and identifying the mechanisms by which cytoskeletal changes may lead to cellular differentiation of a valvular phenotype is a first critical step in enhancing the promotion of a robust valvular phenotype from BMSCs.

Multimodal Optical Spectroscopy Imaging System for in Vivo Demarcation of Epileptic Cortex in Children with Intractable Epilepsy: Laser Doppler Flowmeter

Authors: Diego Colunge, Mohamed Almadi

Major Adviser: Dr. Wei-Chiang Lin





Abstract

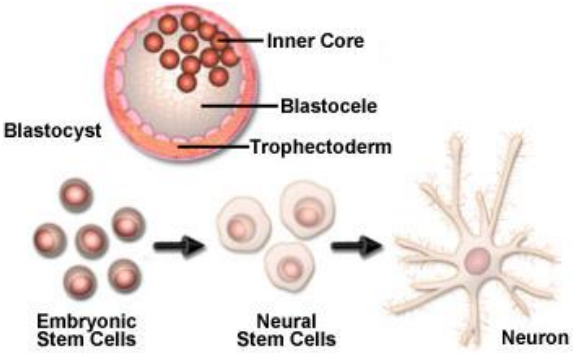
Surgical resection of the cerebral cortex is a well-established treatment for intractable epilepsy in children. The surgical plan is guided by integrating multimodal information from clinical and investigative tests including scalp electroencephalography (EEG), structural imaging, and nuclear medicine studies. The resection boundaries are generally defined by extrapolating extra-operative information onto anatomic landmarks with further assistance of intra-operative neuro-navigation systems in select cases.

Recent developments in optical spectroscopy and imaging technologies offer a new means to accurately characterize tissue in vivo. These technologies provide data of both structural and biochemical elements and the metabolic/oxygenation status of examined tissue. Many of these technologies are portable and can produce characterization results in real-time, which make them suitable for characterize in vivo cortex.
The ultimate goal of this research project is to develop a new optical-based surgical guidance system to tailor cortical resection in children with intractable seizures, with its initial focus on focal cortical dysplasia. In this application, a portable optical spectroscopy-imaging system will be designed and built to measure and identify unique intrinsic characteristics of epileptic cortex. The outcomes of this application will, in turn, become the scientific foundation of the envisioned guidance system for epilepsy surgery.

The Metabolite Approach to Mouse Embryonic Stem Cells: A Low-Cost Technique for Efficient Creation of Motor Neurons

Authors: Xizi Dai, Jared Leichner, Wei-Chiang Lin, Yen-Chih Huang

Major Adviser: Yen-Chih Huang



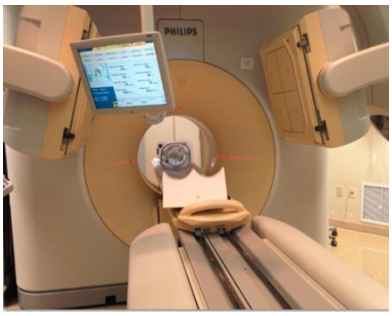
Abstract

Differentiation of functional motor neurons from embryonic stem cells (ESCs) while maximizing viability and efficiency is essential for studying the mechanisms and developing new therapies for motor neuron disease. Currently several methods for motor neuron differentiation have been published, however their efficiency, viability and cost prevents the technology from quickly attaining clinical relevance. Here, we aimed to devise a reproducible protocol to efficiently generate motor neurons by applying four metabolites: Retinoic Acid (RA), Genistein, Sodium Fumarate (NaF) and Sodium Butyrate (NaB). We discovered that with the help of these small molecules, the differentiation efficiency of motor neurons has been dramatically improved. In addition, Immunocytochemistry analysis indicated that motor neuron differentiation was achieved through the enhanced expression of HB9+ ISL1+ and ChAT+. Molecular mechanisms guiding self-renewal and differentiation of stem cells are intricately connected to the metabolic pathways that are activated (or inactivated). While the metabolites we have chosen in our protocol are known to improve resistance to oxidative stress, maximize survival and efficiently induce differentiation, the mechanisms of their action are fundamentally based in metabolic modulation through the down-regulation of glycolysis and up-regulation of oxidative phosphorylation. Using our low-cost novel metabolite method, we were able to selectively modify stem cell metabolism and generate mature motor neurons with improved induction efficiency, speed and viability than existing methods.

DOSIMETRY OF YTTRIUM-90 BREMSSTRAHLUNG SPECT/CT : EXPERIMENTAL PHANTOM STUDY

Authors: Senait A. Debebe, Malek Adjouadi, Juan Franquiz, and Anthony J. McGoron

Major Adviser: Anthony J. McGoron



Abstract

Selective Internal Radiation Therapy (SIRT) involves the administration of ^{90}Y-microsphere via the hepatic artery for the treatment of inoperable liver cancer. Yttrium 90 (^{90}Y) has a half-life of 64.2 hours and decays by the emission of pure beta particles that interact with tissues generating bremsstrahlung photons that have a continuous energy spectrum which can be used for imaging. The biodistribution of ^{90}Y after treatment is generally assessed through bremsstrahlung imaging using SPECT/CT, though the images are of low quality. Information from imaging the distribution of ^{90}Y after SIRT treatment can help to confirm the localization of the microspheres in the tumor and liver as well as serve as a quality assurance procedure to document the absence of pulmonary or extrahepatic gastrointestinal uptake. Post treatment ^{90}Y bremsstrahlung imaging is recommended by most physicians and is currently a routine imaging procedure performed anytime during the first 24 hrs. of treatment.

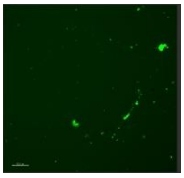
However, the quantitative accuracy of bremsstrahlung SPECT images is limited by the wide and continuous energy spectrum of ^{90}Y bremsstrahlung photons. This continuous nature hinders effective energy based scatter rejection that results in poor image quality due to the presence of the substantial number of photons that are scattered, back scattered and penetrate the collimator speta associated with primary photons. This leads to inaccurate quantitation of microsphere biodistribution especially in small tumors. The goal of this study is to improve the quantitative accuracy of ^{90}Y bremsstrahlung SPECT images and develop a more accurate three dimensional dosimetry method to improve SIRT and ultimately clinical outcomes.

A procedure for Immunohistochemistry on focal epileptic rats

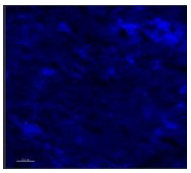
Authors: Abhay Deshmukh, Chelsie Boodoo, Jorge Riera

Major Adviser: Dr. Jorge Riera

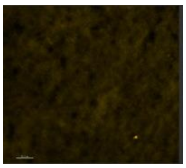
A-1) Old protocol



SF101

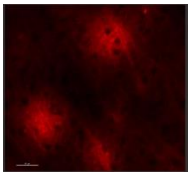


Neurofilaments

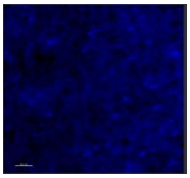


Fluoromyelin

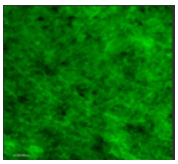
A-2) New protocol



SF101

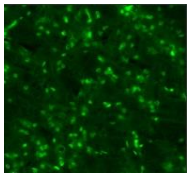


Neurofilaments

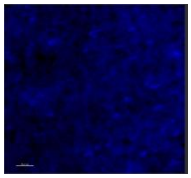


Fluoromyelin

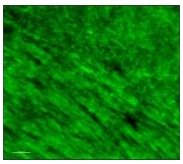
B-1) Anatomical markers



Nissl

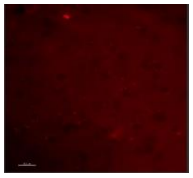


Neurofilaments

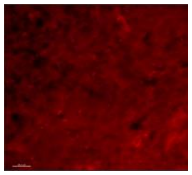


Fluoromyelin

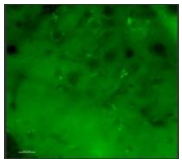
B2) Inflammatory markers



IL-1-beta

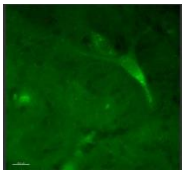


HMGB-I

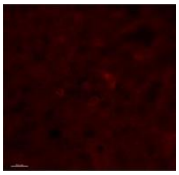


TNF-alpha

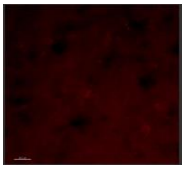
B3) Functional markers



mGluR5

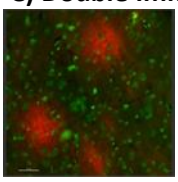


NMDAR2B

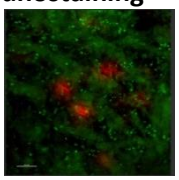


GABA R-alpha6

C) Double Immunostaining



SF101+ Nissl



SF101 + Nissl

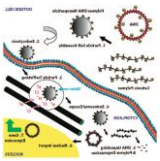
Abstract

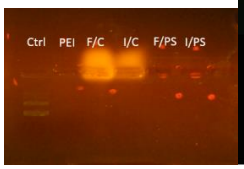
Immunohistochemistry of tissues is a powerful tool used to demonstrate the presence or absence of an antigen. The immunocytochemical staining of brain tissue can be compromised by factors such as time of tissue dissection, type of fixation and storage time in fixative. Sometimes an animal has to go through different research studies which make it difficult to obtain fresh brain tissue, or brain tissue that has been in fixative for a short time for scientific research purposes and may not be available until after several months of fixation. Brain tissues for scientific research are usually fixed by perfusion and immersion in 4% formaldehyde for 24-48 hrs. If fixed for longer times, the antigens are masked by crosslinking of proteins by aldehyde over fixation. Proteolytic enzyme predigestion is often used to unmask the antigens (e.g., Battiflora and Kopinski, 1986) but the results are not satisfactory with over fixed tissue. Shi et al. (1991) described a method of antigen retrieval from formalin-fixed, paraffin-embedded human brain sections based on microwave heating in metal solutions. However no single methodology is available to immunostain all the antigens in rat brains that are fixed for longer times. We demonstrate a procedure to process and immunostain whole brains of focal epileptic rat models that has been fixed for few months to a year. We were successful to immunostain three categories of focal epilepsy specific markers namely, anatomical, inflammatory and functional with fluorescence immunohistochemistry.

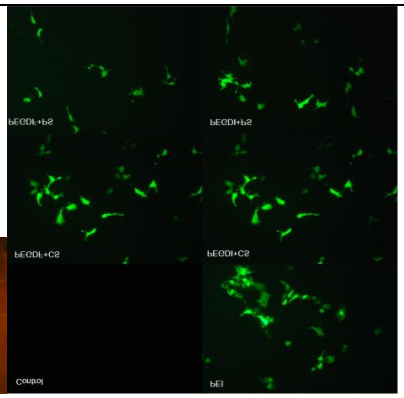
Modified PEGDF and PEGDI Polymers for Non-Viral Gene Delivery in HEK 293 Cells

Authors: Anh Le, Xizi Dai, Y.C. Huang*

Major Adviser: Y.C. Huang







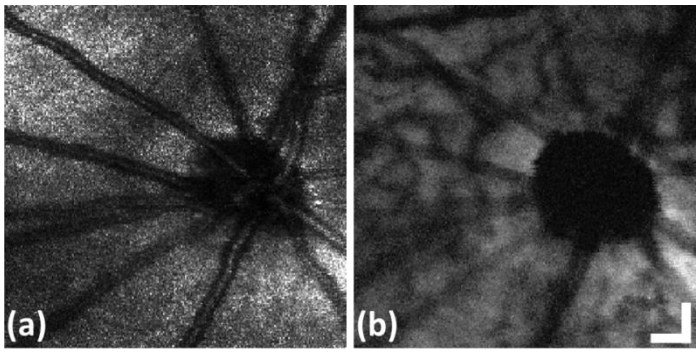
Abstract

Gene therapy involves the use of nucleic acids, either DNA or RNA, for the treatment, cure, or prevention of human diseases. Synthetic cationic polymers are promising as a tool for gene delivery because of their high level of design flexibility for biomaterial construction. Our objective is to develop a polymer based delivery system that would present a safe and effective method for the delivery of DNA. In our lab we have formed a novel polymer of (poly(polyethylene glycol-dodecanoate) (PEGD). PEGD is a polyester of polyethylene glycol (PEG) and dodecanedioic acid (DDA); formation of the polymer is through ester bonds and degradation occurs by hydrolysis. It is a linear viscous polymer and when placed in an aqueous environment self assembles into a vesicle. A copolymer of dodecanedioc acid and polyethylene glycol (PEG) was synthesized at a 1:1 ratio. Furmaric or itaconic acid was used to replace DDA in the polyethylene glycol dodecanedioc (PEGD) copolymer at an 80:20 ratio (DDA: furmaric/itaconic acid). The PEGDF or PEGDI polymers were later modified to incorporate a cationic ligand to introduce a positive charge to the backbone of the polymer. Modified PEGDI and PEGDF are tested to determine if it is capable of binding and condensing DNA.

Optical coherence photoacoustic microscopy for in vivo multimodal retinal imaging

Authors: Xiaojing Liu

Major Adviser: Shuliang Jiao



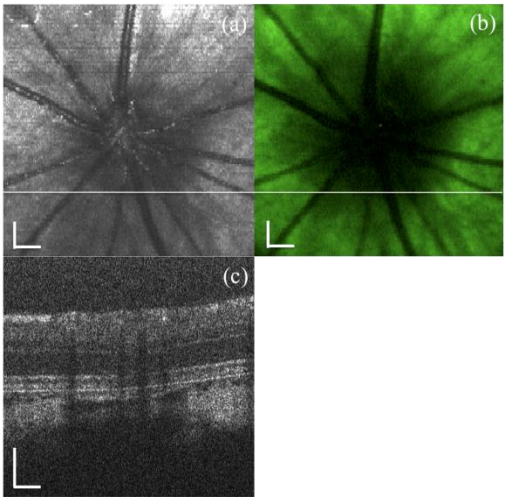
Abstract

We developed an optical coherence photoacoustic microscopy (OC-PAM) system, which can accomplish optical coherence tomography (OCT) and photoacoustic microscopy (PAM) simultaneously by using a single pulsed broadband light source. With a center wavelength of 800 nm and a bandwidth of 30 nm the system is suitable for imaging the retina. Generated from the same group of photons the OCT and PAM images are intrinsically registered. To test the capabilities of the system on multimodal ophthalmic imaging we imaged the retina of pigmented rats. The OCT images revealed the retinal structures with quality similar to conventional OCT while the PAM images revealed the distribution of absorbers in the retina. Since the absorption of hemoglobin is relatively weak at around 800 nm, the NIR PAM signals are generated mainly from melanin in the posterior segment of the eye, thus provide melanin specific imaging of the retina.

Simultaneous optical coherence tomography and lipofuscin autofluorescence imaging of the retina with a single broadband light source at 480nm

Authors: Xiaojing Liu

Major Adviser: Shuliang Jiao



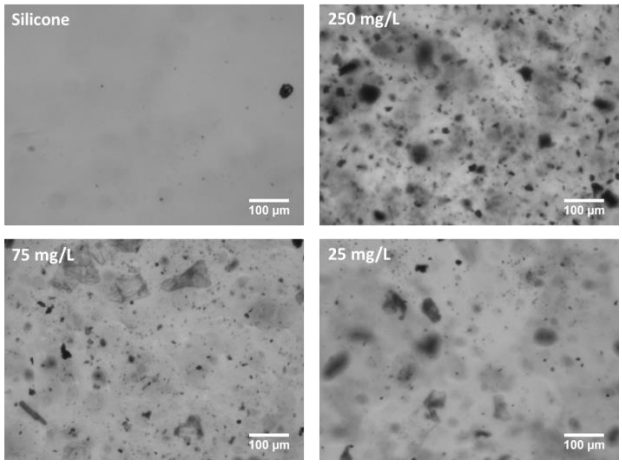
Abstract

We accomplished spectral domain optical coherence tomography and auto-fluorescence microscopy for imaging the retina with a single broadband light source centered at 480 nm. This technique is able to provide simultaneous structural imaging and lipofuscin molecular contrast of the retina. Since the two imaging modalities are provided by the same group of photons, their images are intrinsically registered. To test the capabilities of the technique we periodically imaged the retinas of the same rats for four weeks. The images successfully demonstrated lipofuscin accumulation in the retinal pigment epithelium with aging. The experimental results showed that the dual-modal imaging system can be a potentially powerful tool in the study of age-related degenerative retinal diseases.

Silicone Graphene Composite Material for Heartvalve Applications

Authors: Makensley Lordeus; Angie Estrada; Danique Stewart; Cheng Zhang; Rupak Dua; Arvind Agarwal; Sharan Ramaswamy

Major Adviser: Ramaswamy, Sharan



Abstract

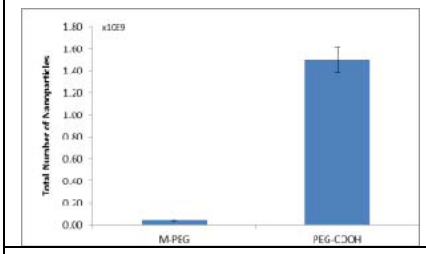
Patients with mechanical heart valves have to take lifelong anticoagulants to prevent risk of thrombus. Emerging, elastomer heart valves have been shown to be able to better recreate the flow physics of native heart valves, resulting in preferable hemodynamic responses. Unfortunately, elastomers, such as silicone, are prone to structural failure due to its poor tear strength which drastically limits their applicability to heart valve prosthetic development. In this study we reinforced silicone with graphene nanoplatelets. Graphene is a high strength material available in many forms.

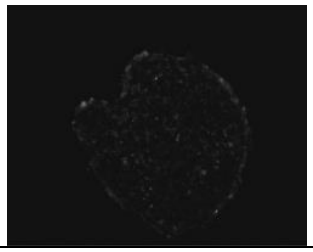
The nanoplatelets were introduced into a 2 part silicone mixture and allowed to cure. Three concentrations of graphene and silicone were tested: 250 mg, 75 mg, and 25mg of graphene per liter of uncured silicone. The mechanical and cytotoxic properties of the graphene-silicone composite material were subsequently characterized using tensile testing and a SRB assay respectively. The introduction of graphene to silicone changed the tensile properties of the elastomer slightly but not in a significant manner ($P > 0.05$). The control sample had a Young's Modulus of 0.70 MPa; the samples containing 250mg, 75 mg, and 25mg of graphene per liter of uncured silicone had a Young's Modulus of 0.85 MPa, 0.67 MPa, and 0.79 MPa, respectively. The SRB assay further showed that the graphene did not in any way inhibit the growth of endothelial cells. We found that 250 mg of graphene significantly improved ($p<0.05$) the material response to fatigue conditions compared to silicone-alone. These findings suggest that for the silicone-graphene composite, static loads were principally transferred onto the matrix; on the other hand, in cyclic loading conditions, the graphene nanoplatelets were recruited effectively to delay failure of the bulk material. We conclude that application of graphene nanoplatelets to extend silicone durability is useful and warrants further evaluation at the tri-leaflet valve configuration.

The Effect of Surface Functionalization and Temperature on Nanoparticle Penetration into Tumor Spheroids

Authors: Abhignyan Nagesetti, Diego Estumano, Helcio R. B. Orlande, Marcelo J. Colaço, George Dulikravich

Major Adviser: Anthony McGoron





Abstract

Nanoparticle characteristics such as size, surface charge and surface groups influence their cellular uptake and transport in tumor interstitium. In poorly vascularized and avascular tumors, the dense packing of cells and tumor extracellular matrix present additional barriers to diffusion of nanoparticles. This leads to a heterogeneous distribution of drugs and limits their applications in diagnostics and therapeutics. At present, strategies to improve the penetration of drugs in avascular tumors are limited. We hypothesized that hyperthermia (at +43 to +45 ℃) will improve the distribution of drugs in avascular tumors by increasing the internalization rate constant of nanoparticles. Furthermore we hypothesized that surface groups on nanoparticles play a significant role in cell uptake and consequently in intratumoral distribution. To test our hypotheses, we formulated fluorescent silica nanoparticles coated with Methoxy- Polyethylene Glycol (M-PEG; 5000 Da) and Carboxy Methyl-Polyethylene Glycol (CM-PEG; 5000 Da) and performed cell uptake studies in ovarian carcinoma cell line (SKOV-3) at different temperatures. A SKOV-3 spheroid model that recreates most of the histomorphological complexity of avascular tumor is used for studying the distribution of nanoparticles. We have confirmed that the temperature and surface modification are critical factors in determining nanoparticle transport in avascular tumors. Spheroid models of ovarian cancer are clinically relevant as late stage metastases. In addition diffusion-reaction model has been formulated to simulate the penetration of nanoparticles.The mathematical model in conjunction with experiments in histomorphologically relevant tumors will assist in the rigorous screening of theranostic carriers and hence can be used to design nanoparticle delivery strategies to *in-vivo* tumors.

Regulation of Vascular Tone via Localized Calcium Signaling Around Myoendothelial Projections

Authors: Jaimit Parikh, Adam. Kapela, and Nikolaos Tsoukias

Major Advisor: Nikolaos Tsoukias

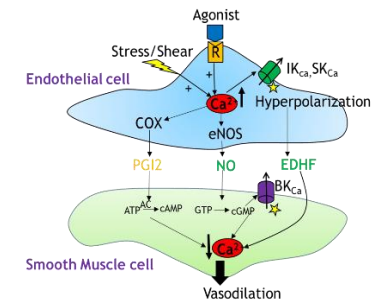


Figure 1: Schematic illustrating the important pathways of EC modulation of SMC contractility

(Sandow et al. 2009)

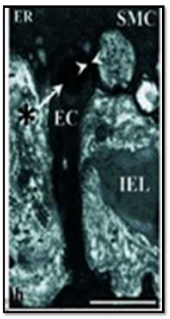


Figure 2: Microprojection structure: Experiment

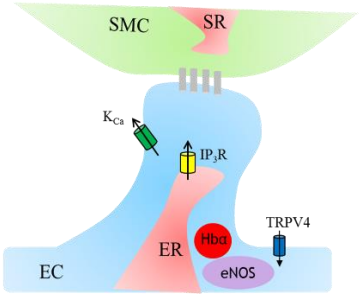


Figure 3 . Schematic of the major cellular components localized in the microprojections

Abstract

Introduction: Localized calcium (Ca^{2+}) events like sparks, puffs and sparklets have often been documented in vascular smooth muscle (SMC) and endothelial (EC) cells. Endothelial membrane projections across the internal elastic lamina, called myoendothelial projections (MPs), bring the membrane of the two cells in close proximity and allow for communication between the two cell layers and the exchange of ions and second messengers. Experimental studies have suggested concentrated presence of intermediate-conductance calcium-activated potassium channels (IK_{Ca}), IP_3 receptors (IP_3Rs), endothelial Nitric Oxide Synthase (eNOS), Hemoglobin alpha (Hbα) and members of the Transient Receptor Potential channel family (TRPV4) in the vicinity of the MPs. Furthermore, localized endothelial Ca^{2+} elevation in the vicinity of MPs has been observed during EC and SMC stimulation. Thus, experiments have begun to unveil the presence of complex machinery at these sites that regulate myoendothelial communication and vasoreactive signaling.

Methods: We utilize detailed computational models of Ca^{2+} and plasma membrane potential dynamics in the SMC and EC.

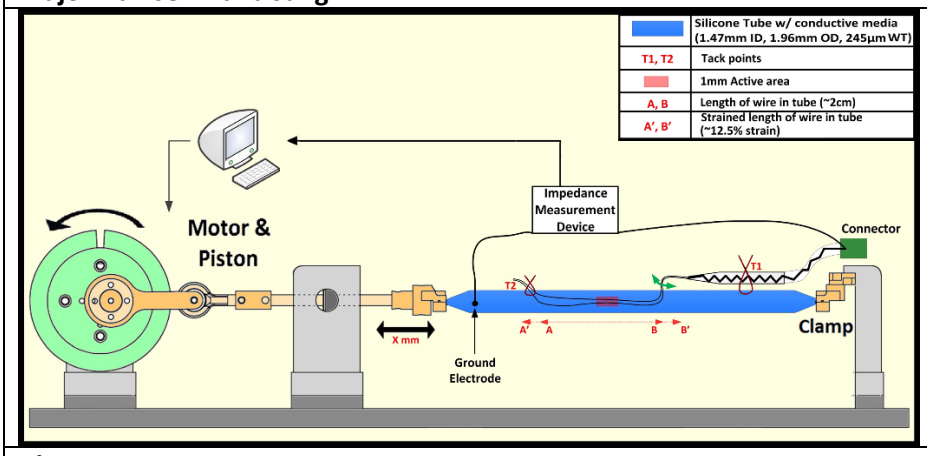
Results and Discussion: Activation of TRPV4 channels results in localized Ca^{2+} increase with typical concentrations around few μM and a spatial spread of few μm. Sparklet characteristics are affected by a variety of factors including channel gating, cooperativity and conductance. These localized Ca^{2+} events can initiate endothelium-derived hyperpolarizations (EDH) and relaxation factors (EDRF) (i.e. by activating local IK_{Ca} channels or eNOS) modulating vascular tone. Localization of eNOS in the vicinity of MP resulted in NO mediated feedback during SMC stimulation. Modulation of NO biovailability by Hbα is enhanced by their co-localization in the MP. The model makes predictions for the required Hbα concentration for modulation of NO signaling.

Conclusions: Detailed models of Ca^{2+} and membrane potential dynamics in vascular cells explores the role of localized Ca^{2+} events in the vicinity of MPs and the regulation of vascular tone.

Fatigue testing of longitudinal intrafascicular electrodes as a peripheral nerve interface

Authors: Andres Pena, Sathyakumar S. K., James Abbas and Ranu Jung

Major Adviser: Ranu Jung



Abstract

Peripheral nerves are exposed to considerable stresses during routine activities. For peripheral nerve interfaces, the design and deployment of the device will influence the stress that it will experience and mechanical fatigue testing should be used to assess the long-term reliability of the device components.

The longitudinal intrafascicular electrode (LIFE) can be used to stimulate or record from small groups of axons in peripheral nerve fascicles. In our laboratory, each LIFE is made from 25 µm, polymer insulated Pt/Ir wire (lead) with an exposed area (electrode) to electrically interface with the axons. Using an attached needle, the electrode is sewn longitudinally into the fascicle to place the exposed area within the fascicle, the needle is removed, and then the lead is sutured to the nerve bundle to anchor it near the entry and exit points. In this work, we have developed a test setup to evaluate the susceptibility of these leads to damage near the anchor points, which are potential locations of high stress.

The test setup was designed to mimic the key features of the anchored lead: the wire is anchored at two sites along a compliant structure that may undergo substantial repetitive, longitudinal strain. The test setup consists of a small silicone tube (Nusil, 1.47mm ID, 1.96mm OD) that is filled with a conductive solution (2% agarose in 0.9% saline), fixed at one end and attached to a piston at the other. The piston mechanism uses a software-controlled brushless motor coupled to a linear motion assembly to apply cyclic strain to the tube to a level that mimics physiological nerve strain conditions (e.g. upper arm nerves can tolerate up to 8% strain). Electrodes are inserted into the tube and anchored with suture at the entry and exit points. For continuous impedance monitoring and recording, a tungsten needle welded to a wire is installed in the tube as a ground electrode. In order to prevent leakage of the conductive solution and introduction of air bubbles into the tube, a thin film of petroleum jelly is applied around it. An optional test configuration could use a heated saline bath to keep the tube and agarose moist and to facilitate accelerated testing protocols. To-date, this setup has been used to demonstrate that a LIFE maintains electrical continuity after more than 1 million cycles of 12.5% simulated nerve strain. Future testing will investigate additional samples for a larger number of cycles.

This work was supported by NIH-R01-EB008578.

Mechanical conditioning for valvulogenesis in tissue engineered heart valve

Authors: Sasmita Rath, Ana Villegas, Manuel Salinas

Major Adviser: Dr. Sharan Ramaswamy

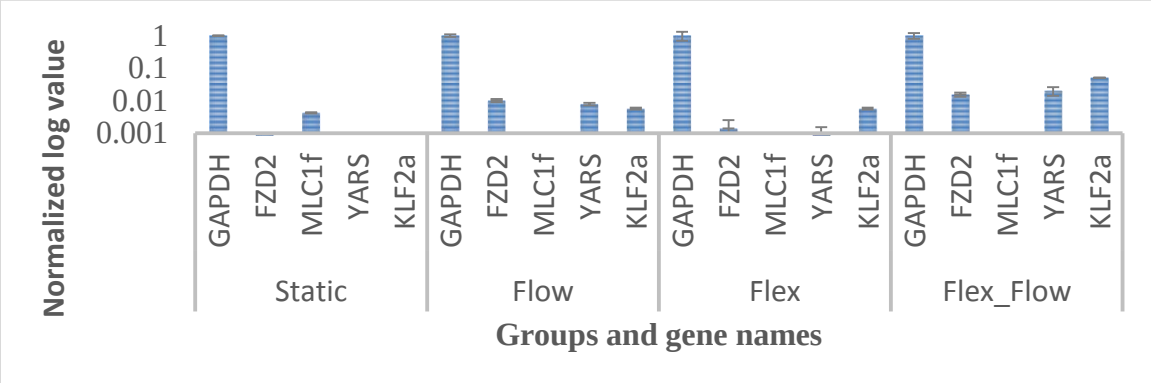


Figure 1: Quantitative Real Time Polymerase Chain Reaction (QRT-PCR) results: absence of MLC1f and presence of FZD2 indicates a valve SMC type differentiation, high expression of YARS indicates a valve EC type differentiation, whereas significant expression of klf2a ($p<<0.05$) confirms a valve phenotype in flex_flow group

Abstract

Fluid shear stress is critical for heart valve (HV) development, maintenance and functioning. Previous studies suggested that combining effects of flow and cyclic flexure drastically improved extra cellular matrix (ECM) component, such as collagen production under sub-physiological fluid flow conditions [i]. Here we sought to understand the role of physiological shear stress and flexure effects on *de novo* HV tissue formation and resulting gene and protein expression.

A bioreactor system, bone marrow mesenchymal stem cells (BMSCs) seeded on polymer scaffolds were used for experimentation. Control, Flow, Flex, Flex_Flow groups were exposed to no flow and no flex, steady flow imparting shear stresses in the physiological range of 5 to 6 dynes/cm^2, cyclic flexure at a physiological frequency of 1 Hz (equivalent to a heartbeat rate of 60 bpm), both physiological shear stress and frequency respectively for 14days.

Collagen content was significantly higher in flex_flow group. Additionally, gene expression study among all groups suggests that flex_flow group express significant klf2a and high expression of YARS during tissue regeneration. Computational study in our laboratory reveals that cyclic flexure group alone imparts a large degree of localized oscillatory shear stress (OSS) and flow group experience steady shear stress (SS); whereas both OSS and SS magnitudes were detected in flex_flow group. This suggests that the driving factor in flow group is shear stress, in cyclic flexure is OSS, whereas both parameters are present in combined flow and flexure group.

Therefore, we conclude that the combination of steady flow and cyclic flexure helps support engineered tissue formation as previously observed, by the co-existence of both OSS and appreciable shear stress magnitudes, and potentially augment valvular gene and protein expression when both parameters are in the physiological range.

References:
i Engelmayer GC; Biomaterials 27 (2006) 6083–6095
ii Vermot J; PlosOne 7 (2009) e1000246-e1000246

Heart Valve Tissue Engineering: A Study of the Effects of Sample Movement on the Flow Physics and Mass Transport

Authors: Manuel Salinas[1], Vinu Unnikrishnan[2], Sharan Ramaswamy[1]
[1]Department of Biomedical Engineering, Florida International University, Miami, FL
[2]Department of Aerospace Engineering and Mechanics, The University of Alabama, Tuscaloosa, AL
Major Adviser: Dr. Sharan Ramaswamy

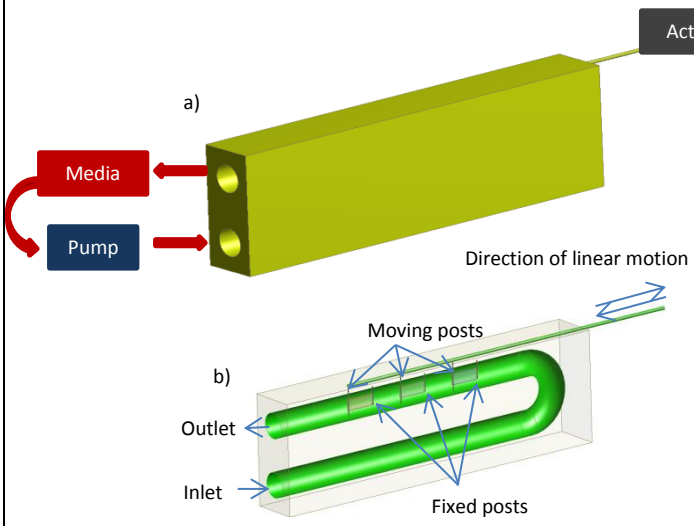


Figure 1. a) Single bioreactor chamber. An external actuator attached to one end of the samples provides movement. b) Inside look of the bioreactor. Each sample has one end fixed while the other is allowed to move linearly by means of the actuator.

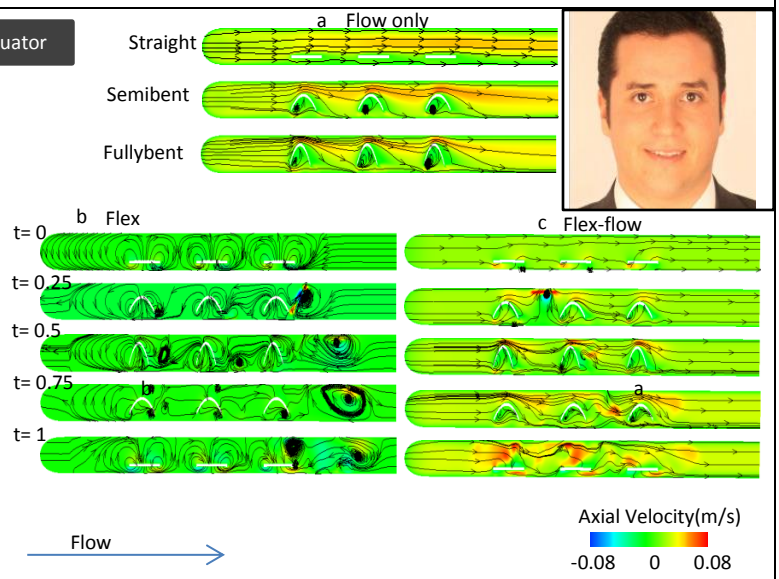


Figure 2. Contour of velocity magnitudes and velocity streamlines in the plane (x= 17.5 mm) at the center line of the specimens for cases of a) flow only, b) flex only, and c) flex-flow.

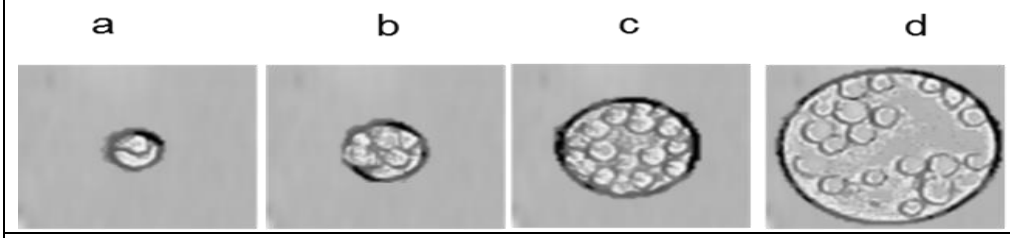
Abstract

Mechanical conditioning has been shown to promote tissue formation in a wide variety of tissue engineering studies, but the underlying mechanisms by which external mechanical stimuli regulate cells and tissues are not fully understood. This is particularly relevant in the area of heart valve tissue engineering (HVTE) due to the intense hemodynamic environments that surround native valves. Some studies suggest that oscillatory shear stress (OSS) caused by steady flow and scaffold flexure play a critical role in engineered tissue formation derived from bone marrow derived stem cells (BMSCs). In addition, scaffold flexure may enhance the transport of nutrients such as glucose and oxygen. In this study, we computationally quantified the i) magnitude of fluid-induced shear stresses; ii) the extent of temporal fluid oscillations in the flow field using an oscillatory shear index (OSI) parameter, and iii) glucose and oxygen mass transport profiles. We incorporated moving boundary computational fluid dynamic (CFD) simulations of samples housed within a bioreactor (Fig.1) to consider the effects of: 1) No flow, no flexure (control group), 2) Steady flow-alone, 3) Cyclic flexure-alone, and 4) Combined steady flow and cyclic flexure environments. In addition, we coupled a convective-diffusive mass transport equation to the CFD simulations. We found that the coexistence of both OSS and appreciable shear stress magnitudes explained the high levels of engineered collagen previously observed from combining cyclic flexure and steady flow states. On the other hand, each of these metrics on its own showed no association. This finding suggests that cyclic flexure and steady flow synergistically promote engineered heart valve tissue production via OSS, so long as the oscillations are accompanied by a critical magnitude of shear stress. Oxygen and glucose mass distributions were also enhanced by specimen movement when coupled to scaffolds of significantly low porosity.

MEMS Sensing-Array for Real-Time Nanotoxicity Assessment

Authors: Pratikkumar Shah

Major Adviser: Chenzhong Li



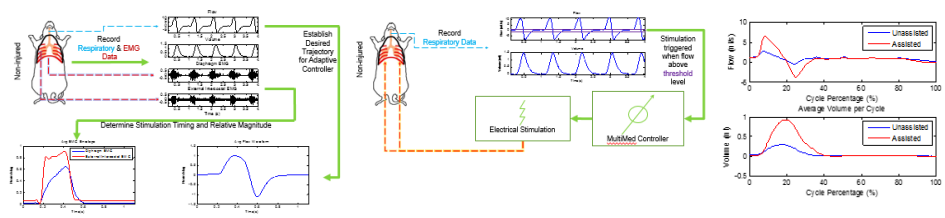
Abstract

Measurement of nanomaterial toxicity is a complex task as the toxicity varies widely based on material composition, size, surface properties and target organ/cell-lines. Traditional nanotoxicity assays on large cell population may hide the important heterogeneity of individual cells often found in neuronal, cancerous and stem cells. The development in the area of new nanomaterial discoveries tracks far ahead compared to the development of advanced tools to measure these materials' toxicity for appropriate usage. It is in demand to develop alternative approaches to assess nanomaterials toxicity rapidly, reliably and accurately. Here, we presented a comparative study of a single cell and a small cell-population in order to chart out the difference in behavior. We demonstrated that a small cell-population offered higher sensitivity and response time for toxic exposure compared to a single cell. This study is very helpful comparison that when capturing and analyzing a single cell is difficult, a small cell-population (less than 20 cells studied here) with appropriate sensing electrode would provide liberty of capturing multiple cells with higher sensitivity and improved response time.

Assistive Respiratory Pacing of the Diaphragm in the Rat Model Based on Ventilatory and Electromyographic Recordings

Authors: Ricardo Siu, Brian Hillen, Brett Davis, Adeline Zbrzeski, Yannick Bornat, Jonathan Castelli, James Abbas, Sylvie Renaud, Ranu Jung

Major Adviser: Ranu Jung



Abstract

Respiratory pacing can be used to provide an alternative to mechanical ventilation for individuals with high cervical spinal cord injury. Although the technique is sometimes successful, incorporating neuromorphic control strategies may provide improved ventilation and promote widespread use. We are currently developing a system to electrically stimulate respiratory muscles, while adapting to changes in electrode properties and physiological demands, to provide sufficient ventilation to the user. A rodent model for ventilatory measurement and assistive stimulation was developed to evaluate an adaptive controller for respiratory pacing. We present results of experiments designed to quantify the physiological response to respiratory challenges and we demonstrate the feasibility of electrically assisted ventilation in this model.

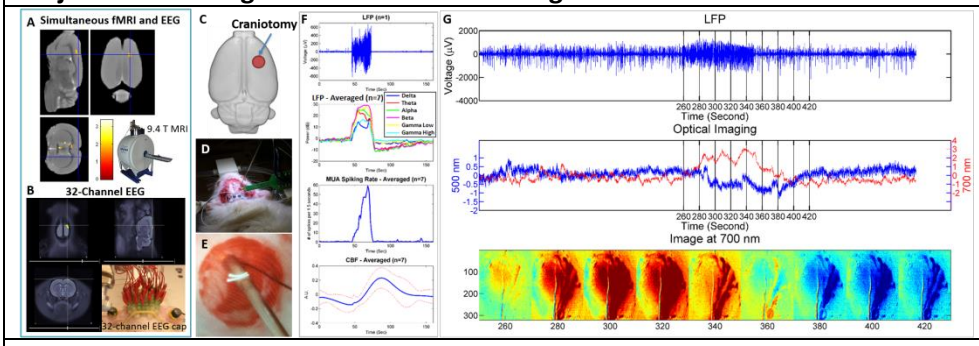
A pneumotachometer and a capnograph were incorporated in a breathing circuit with the animal subject to determine flow, lung volume, and peak end-tidal CO_2. Muscle activation parameters were obtained via intramuscular electromyogram (EMG) recordings from diaphragm and external intercostal muscles. Inspiratory period (T_i), determined from the flow data, remained constant among trials, but expiratory period (T_e) decreased at high ventilatory demands. Peak breath volume, peak inspiratory and expiratory flow, and EMG magnitude increased with elevated respiratory demand. These parameters serve as the target ventilatory response for in vivo testing of the adaptive controller. A respiratory assist stimulation protocol was performed using custom designed stimulation hardware. Diaphragm activation time from EMG data was used to determine the stimulation pulse train duration. Stimulation bursts of 75 Hz were triggered at the onset of inspiration. Cycles with assistive stimulation showed increased breath volume when compared with unassisted breaths. Future work will utilize these results to determine experimental conditions and paradigms for evaluation of a neuromorphic control system in this rodent model.

Supported by R01-NS086088.

Electrophysiological and hemodynamic signatures of epileptic neocortex in rats with focal cortical dysplasia: Implications on epilepsy surgery

Authors: Yinchen Song, Rafael A. Torres, Jihye Bae, Abhay Deshmukh, Wei-Chiang Lin and Jorge J. Riera

Major Adviser: Jorge J. Riera and Wei-Chiang Lin



Abstract

The success of a surgical intervention for intractable epilepsy hinges upon accurate localizations of the epileptic focus. Currently EEG-fMRI recording is considered as a promising tool in localizing both superficial and deep epileptic foci. To date, EEG-fMRI studies have demonstrated that interictal epileptiform discharges (IEDs), the hallmark of epilepsy, can be used to localize the blood-oxygen-level dependent (BOLD) activation as well as deactivation, named irritative zone, which may coincide with the epileptic focus. The relevance of IED-related BOLD response to epileptogenesis is still in debate.

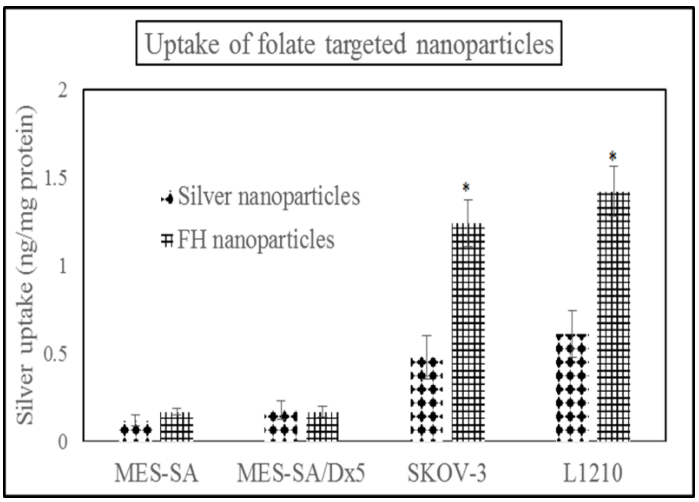
In this study, we performed simultaneous EEG-fMRI recordings on a preclinical model of focal cortical dysplasia (FCD) and compared the localizations of IED-related BOLD responses with results from traditional scalp EEG source imaging. To validate the accuracy of source localizations based on EEG-fMRI and its underlying mechanism with regard to epileptiform discharges, intracranial electrophysiological and hemodynamic recordings were performed on the suspicious irritative zone that was predetermined by both techniques. Scalp EEG source imaging was also conducted alone, as a comparison in efficiency, to provide source information for the intracranial recordings of cortical epileptiform dynamics.

We found that simultaneous EEG/fMRI and multi-channel scalp EEG recordings together provides efficient source localization information of paroxysmal electrical activities in focal epilepsy and they produce findings with a good concordance. This is further verified with both subcutaneous and intracranial electrophysiological recordings on the preclinical model of FCD, as we could see a good match of IEDs between them. Furthermore, six rats experienced ictal periods during the intracranial recordings, which originated from the suspicious irritative zone. Reflections of seizures within the optical field of view resembled an increase of CBV and a decrease of [dHb] with an epicentric origin followed by a peripheral propagation. An unexpected slight decrease in CBF prior to the seizure onset was also spotted. Contributions of electrical activities from different frequency bands to the local hemodynamic response were also evaluated, which indicated a higher correlation between the gamma-low band and CBF response.

Multifunctional silver nanoparticles for targeted cancer therapy

Authors: S Srinivasan, V Bhardwaj

Major Adviser: A J McGoron



Abstract

Noble metal nanoparticles have received increased attention due to their unique optical properties. Gold and silver nanoparticles are widely being used for drug deliver due to their high surface plasmon resonance which enhances the scattering of the molecules in their vicinity also known as Surface enhanced Raman Spectroscopy. Silver nanoparticles have shown to produce higher SERS enhancement of molecules in their vicinity; in comparison to gold nanoparticles.

Folic acid (FA) is an important ligand to folate receptors highly overexpressed in cancer cells and Doxorubicin is a widely used chemotherapeutic agent. Folic acid conjugation and Doxorubicin (DOX) conjugation onto silver nanoparticles help deliver DOX in targeted fashion into cancer cells. Further, exposure of these multifunctional nanoparticles to NIR laser helps enhance their cytotoxicity due to ROS generation.

Measurement of Retina Vascular Flow across Small and Large Vessel Sizes

Authors: Stephen Winhoven , Jessica C. Ramella-Roman

Major Adviser: Dr. Jessica Ramella-Roman

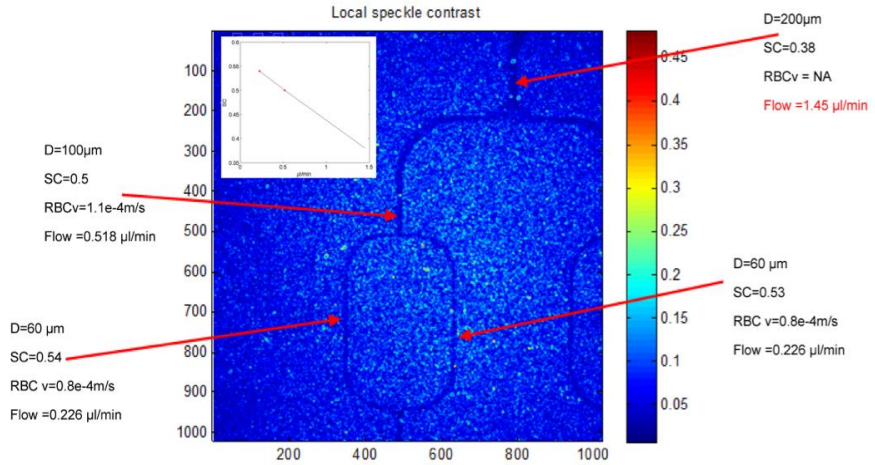


Fig. 1 RBC tracking at small vessels (Diameter 60µm and 100µm) and self-calibration of flow for large vessels (Diameter =200µm)

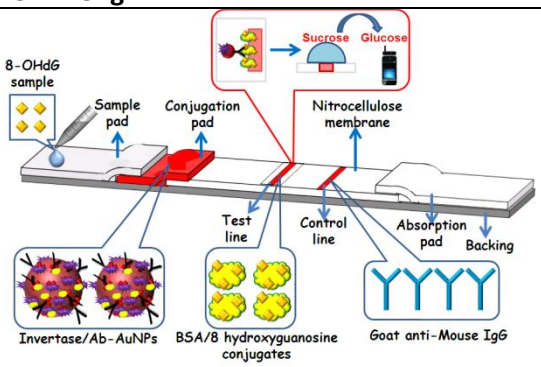
Abstract

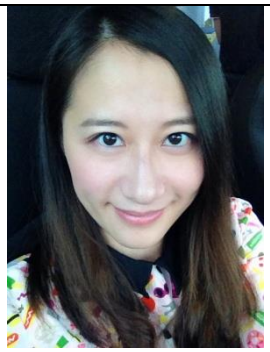
The quantitative estimation of blood flow is an important tool for exploring the etiology, and the treatment of many diseases of the eye including diabetic retinopathy. Current imaging technique focus on limited ranges of vessel sizes. We introduce a novel retinal flow imaging system that combines Speckle Imaging and TRacking of Red blood cells (SITAR). The RBC tracking method is capable of dealing with very low signal-to-noise ratios, relies on tracking the non-uniform distribution of red blood cells within the vessel, rather than the individual blood cells themselves. However, the highly absorptive properties of the RBC's constrain this technique to smaller vessels. Speckle Contrast imaging is capable of measuring velocities across a wide range of vessel diameters, but can only provide a relative measure of speed. The SITAR system combines these techniques and provides not only quantitative values of flow but does so across multiple vessels sizes (250 µm to 20 µm in diameter).

Wireless-enabled Personal Glucose Meter Coupled with Lateral Flow Immunostrip for Quantitative Detection of Non-glucose Target

Authors: Xuena Zhu

Major Adviser: Chen-zhong Li





Abstract

In recent years, much effort has been devoted toward developing point-of-care (POC) devices. Among them, paper-based POC devices is a special category due to the advantages of simple, rapid, on-site, and cost-effective, and has been widely used in home healthcare and medical testing. Lateral flow strip is one of the simplest and most popular formats of paper-based POC devices, and can be used to detect specific substances in a sample by using an immunological reaction. Personal glucose meters (PGMs) is one of the most successfully commercialized diagnostic devices on the market, and it has been widely used by millions of diabetes patients. Generally, glucose meter is capable of detecting glucose as the unique target. To realize non-glucose target detection using a PGM, the relationship between target recognition and glucose generation must be established. Here, we describe a novel design that combines the traditional lateral flow strip with a commercialized PGM for quantitative detection of non-glucose target. The concept was demonstrated by using an oxidative DNA damage biomarker, 8-hydroxy-2'-deoxyguanosine (8-OHdG). The basic design of the device was adapted from our previously reported colorimetric visual detection platform which is based on gold nanoparticles based competitive immunoassay. However, visual detection can provide only qualitative and semi-quantitative results. Thus, to enable quantitative analysis, we establish a novel method that transforms the detection of the target to the detection of an enzyme invertase. The enzyme converts sucrose into glucose for glucose meter readout. The device was able to detect 8-OHdG concentrations in PBS as low as 0.14 ng mL^{-1} with a dynamic range of 0.1-100 ng mL^{-1}. Considering the inherent advantages of the PGM, the demonstration of this device therefore should provide new opportunities for the monitoring of a wide range of biomarkers as well as various target analytes in connection to different molecular recognition events.

FIU | Biomedical Engineering

About the Keynote Speakers:

David E. Drew, Ph.D.
Joseph B. Platt Chair and Professor of Education, Claremont Graduate University
Dr. Drew's research interests focus on the improvement of STEM (Science, Technology, Engineering, and Mathematics) education and research. His book about reforming STEM education, STEM the Tide, is published by the Johns Hopkins University Press. He also conducts research and writes about higher education, and, to a lesser degree, about technology and health/health education. His current projects address: closing the STEM achievement gap, STEM curriculum reform, preparation of excellent STEM teachers, and the integration of engineering education and the liberal arts.

Joseph Culver, Ph.D.
Associate Professor, Washington University School of Medicine
Functional neuroimaging of healthy adults has enabled mapping of brain functions and revolutionized cognitive neuroscience. However, traditional functional brain scanners (e.g., fMRI) are limited to single "snap shots" and provide very limited assessment during rapidly evolving brain injury. Optical imaging has long held promise as a bedside neuroimaging technique. However, image quality has been lacking, particularly in comparison to the gold standard of fMRI. Moreover, traditional functional mapping requires subjects to perform tasks, which is very limiting in clinical populations. This talk will discuss current challenges in high-density diffuse optical tomography, including the development of large field-of-view photonics instrumentation, imaging arrays, and anatomical light modeling. The implications of the new technology for mapping of higher-order, distributed brain function such as language processing and resting-state networks will be discussed.

Program:

10:00 am	Seminar: David E. Drew, Ph.D. (EC 2300)
12:00 pm	Graduate Student Poster Presentation (Panther Pit)
4:00 pm	Seminar: Joseph Culver, Ph.D. (EC 2300)
5:30 pm	Award Ceremony (EC 2300)
6:00 pm	Reception (Panther Pit)

For more information:
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