Bioengineering Research Grants (BRG) New Awards Fiscal Year 2003

PI Name	Institution	Title	Project	Years Awarded	Funding Institute
Andreadis, Stelios T	State University Of New York At Buffalo	Retroviral Gene Transfer To Epidermal Stem Cells	EB000876	5 years	NIBIB

Abstract: The current project seeks to understand retrovirus-mediated gene transfer and to enhace the transduction efficiency to epidermal stem cells. Although retroviral transduction results in permanent genetic modification, differentiation and eventually loss of the transduced cells from the epidermis results in temporary transgene expression. Therefore, to achieve stable long-term gene expression, it is critical that the epidermal stem cells are transduced with high efficiency. Recent results from our laboratory show that gene transfer on fibronectin is significantly higher than on tissue culture plastic and implicate integrins in retroviral transduction of epidermal keratinocytes. Specifically, the efficiency of gene transfer correlates with the levels of alpha 5 and beta 1 integrins on the cell surface and blocking these integrins with antibodies decreases gene transfer significantly. We hypothesize that integrins may enhance gene transfer by enhancing the rate of keratinocyte migration on fibronectin and therefore the probability that itarget cells encounter the immobilized virus. We propose to develop two assays to test this hypothesis and use flow cytometry to identify other integrins that may also play a role in retroviral gene transfer. Since integr ns and attachment to extracellular matrix have been shown to characterize keratinocyte stem cell phenotype, we also propose to test the hypothesis that retroviral transduction on fibronectin increases the efficiency of gene transfer to epidermal stem cells. To this end we will use biochemical (e.g. flow cytometry) and functional assays (e.g. clonal analysis), as well as in vivo transplantation of genetically modified skin equivalents onto athymic mice. Finally we propose to engineer micropatterned surfaces and microfluidic networks to enhance deposition of retrovirus to the surface. Mathematical modeling and experiments will be employed to design the gene transfer "chips" in order to increase the efficiency of gene transfer and control the number of gene copies per target cell. Reaching these goals will have significant impact on the potential of genetically modified cells to treat short or long-term disease states.

PI Name	Institution	Title	Project	Years Awarded	Funding Institute
Bellamkonda, Ravi V	Case Western Reserve University	Strain-Induced Scarring & Its Effects On Microelectrodes	NS045072	5 years	NINDS

Abstract: Silicon microelectrode array technology holds considerable promise in advancing the goal of developing stable, electrode-brain interfaces. Chronic unit recordings from multiple neurons in the brain would significantly enhance our understanding of normal physiology and provide a valuable control signal for use in neuro-prosthetic devices. However, the current generation of silicon microelectrodes does not allow stable long-term recordings. The precise mechanisms that cause failure of silicon microelectrode mediated recordings are not known. We hypothesize that the million-fold stiffness mismatch between silicon and neural tissues generates shearing forces at the interface resulting in an astro-glial scar formation that progressively excludes neurons from the vicinity of the recording electrodes. To test our hypothesis, we propose novel and innovative methods to a) determine the strain-sensitivity of primary astrocytes in terms of their adopting a scarring phenotype; b) determine if strain-induced scar formation around Si-microelectrodes degrades their recording capabilities in organotypic hippocampal slice cultures; and c) design coatings for Si-microelectrodes that allow the sustained local release of anti-inflammatory agents to decrease scarring and increase recording stability. The above aims represent a highly inter-disciplinary investigation of an important problem in the design and development of stable neuro-prosthetic devices. Successful completion of our research goals is likely to impact the mechanical and biochemical aspects of the design of the next generation of silicon microelectrode arrays, and subsequently significantly impact the quality of life of persons with disabilities.

Bhatia, Sangeeta N   University Of California San Diego   Intercellular Communications In Hepatic Function   DK065152   4 years   NIDDK

Abstract: This project focuses on development and characterization of an in vitro model of hepatic tissue by control of cell-cell interactions. The difficulty in sustaining differentiated hepatocyte functions in vitro has negatively impacted progress towards cell-based therapies for liver disease as well as in vitro experimentation (e.g. drug toxicity studies). It is proposed that an integrated 'biomimetic' platform incorporating key hepatic features (differentiated hepatocytes, compartmentalized metabolism, oriented cell-cell interactions, and directional fluid flow) will serve as a better predictive platform than existing models for Xenobiotic metabolism and physiological experimentation. Preliminary data suggest that co-cultivation of hepatocytes with non-parenchymal cells (fibroblasts) results in long-term differentiated functions, though neither the molecular basis for the 'co-culture effect' nor the dynamics of the process are well understood. In the proposed research, we aim to characterize the dynamics of the co-culture response, uncover the mechanisms that underlie the co-culture response, and incorporate the required elements in a microfabricated array of bioreactors that mimics features of the liver in a high-throughput platform. Specific Aim 1 will be to investigate the dynamic role of homotypic hepatocyte/hepatocyte) gap junction communication and heterotypic (hepatocyte/fibroblast) contact on differentiated functions. The investigator has developed a micropatterning tool that enables control of cell-cell interactions. Electroactive micropatterned surfaces will be utilized to dynamically release fibroblasts from co-culture and study the impact on hepatic function. The mechanism of the 'co-culture effect' was investigated previously using gene expression profiling of various fibroblast strains. Preliminary results indicate that cadherins may play a role in heterotypic signaling. Specific Aim 2 will be to investigate the role of cadherins in coculture, in particular T-cadherin, a candidate that was differentially expressed by over 30-fold. Preliminary results indicate that compartmentalized functions of the liver can be recreated in vitro using controlled oxygen gradients. Specific Aim 3 will be to combine oxygen gradients and directional fluid flow with differentiated hepatocytes (as determined in SA1 & 2) into an array of miniaturized bioreactors that can be used as predictive models of the liver. The engineered tissue will be assessed by examining the responses to well-characterized stimuli. This project will lead to an integrated understanding of how cell-cell interactions produce coordinated organ function and will establish a robust predictive model of liver function for pharmaceutical drug development and fundamental hepatic studies.

Bolch, Wesley E   University Of Florida   Advances In Skeletal Dosimetry Through Microimaging   CA096441   4 years   NCI

Abstract: Toxicity of the hematopoietically active bone marrow continues to limit the amount of radioactivity that can be delivered to patients undergoing radioimmunotherapy. High-activity administrations thus demand interventional procedures, while low-activity administrations avoid marrow toxicity but at the potential cost of suboptimal therapy. Accurate dosimetry of the active bone marrow provides the best indicator of marrow toxicity, but only if the dose estimate is highly patient specific. This level of specificity requires separate assessments of activity uptake in the patient's skeletal tissues, as well as the proper selection of radionuclide S values. S values for skeletal dosimetry are currently taken from a Reference Man model that utilizes 30- year-old optical scanning data of a single male subject. Skeletal mass data come from separate and independent sources, with key studies published in 1926. Recent studies using NMR microscopy of the femoral head and humerus have shown that scaling of Reference Man to female subjects is poor and inconsistent. Accurate scaling of S values is based on measurements of skeletal volumes in both a reference individual and in the patient. Consequently, the project Specific Aims are: (1) to construct a detailed and comprehensive reference skeletal model for the adult male and female using cadavers of nominal body mass index and an age representative of radionuclide therapy patients. /nformation on in-vivo skeletal structure will be made via whole-body CT. Detailed dosimetry for all major skeletal structures will be accomplished through bone site harvesting, sectioning of spongiosa, imaging of the trabecular microstructure through either NMR microscopy or mieroCT, and radiation transport modeling; (2) to verify methods of scaling S values to specific patients using CT analyses of skeletal structure in patients scheduled for total hip arthroplasty. Recovery and NMR microscopy of the excised femoral heads will permit final verification of scaled patient marrow dosimetry; (3) to assess the degree to which ratios of spongiosa volumes between different individual varies among difference skeletal sites. Additional tasks will include the construction of intra-skeletal-site dose-volume histograms of marrow dose, improved models of the trabecular surfaces, and improved dosimetry for alpha-emitters in radionuclide therapy utilizing the NMR microscopy and microCT images.

Brunski, John	Rensselaer Polytechnic Institute	Mechanobiology At Healing Bone-Implant Interfaces	EB000504	4 years	NIBIB

Abstract: Immediately loaded orthopaedic and oral implants can undergo interfacial micromotion and fail by forming interfacial fibrous or cartilaginous tissue rather than bone. While in this instance and others it is evident that mechanical conditions affect healing of skeletal tissue ("mechanobiology"), specific factors influencing cell fate decisions, and mechanisms by which cells interpret and respond to changes in mechanical environment, remain unclear. This gap in understanding restricts design of conventional as well as new tissue-engineered implants. However, recent insights from fracture healing and other healing situations suggests the hypothesis that extracellular matrix remodeling and angiogenesis are central in bone healing around implants, and that tissue deformation (strain) during early implant loading affects these processes, and, in turn, cell differentiation at interfaces. To test this idea, this project will measure spatial/temporal expression of key molecular makers of angiogenesis, bone, and cartilage formation at: 1) healing sites without implants: and 2) healing interfaces where biomechanical strain conditions are varied. The project will use a novel model of the bone-implant interface in mouse tibiae, which allows experimental control of biomaterial and biomechanical factors. Also, the project will use wild-type (wt) and NEMP9-null mice (MMP9-/-), since the latter lack a key matrix metalloproteinase (MMP9, gelatinase B) involved in angiogenesis. The project will integrate data from in situ hybridization, ultrastructural analyses, immunocytochemistry and biomechanical tests to examine mechanobiology of interfacial healing. Aim 1 will test if matrix remodeling and angiogenesis are prerequisites for osteoblast (OB) differentiation in holes without implants; the aim will develop a "molecular map" of expression of markers of angiogenesis, bone and cartilage formation during healing of empty drill holes (diam. 0.2, 0.4 and 1.0 ram) at 3, 9 and 27 days in wt and MMP9-/- mice. Aim 2 will test if matrix remodeling and angiogenesis are essential for differentiation of mesenchymal cells into OBs in unstrained bone-implant gaps. Using wt and MMP9-/- mice, we will develop molecular maps of healing at 0, 3, 9 and 27 days at: (1) a bone-implant gap interface (BIGI) around stabilized polylactide (PLA) pins in oversized holes; and (2) a direct bone-implant interface (DBII), having a mix of direct bone-implant contact and gaps around stabilized PLA screws. Aim 3 will control implant micromotion in its implant bed, in response to a defined load immediately post-implantation, to test whether specific strain conditions in interfacial gaps inhibit matrix remodeling, angiogenesis and differentiation of mesenchymal cells into OBs. A miniaturized rmcromotion device will be used to create implant stability vs. cyclic axial implant micromotion (100-200 (m) of pins and screws in a BIGI during healing. Interfaces will be analyzed biologically as in Aims 1-2, and interfacial strain fields will be measured directly by digital image correlation based on micro-CT images of whole bone-implant specimens.

Dennis, Donn M	University Of Florida	Local Anesthetic Cardiotoxicity: Nanotechnology Therapy	GM063679	4 years	NIGMS

Abstract: Local anesthetics (LAs) reversibly prevent impulse transmission in nerves by voltage-, time- and frequency-dependent blockade of sodium channel conductance (I-Na). These pharmacological actions underlie the therapeutic use of LAs in clinical care to provide regional anesthesia (e.g., epidural blockade) for patients undergoing surgical procedures or childbirth. However, inadvertent intravascular injection or overdose may lead to undesired INa blockade in other tissues (e.g., heart, central nervous system) and thereby cause potentially life threatening adverse events (e.g., cardiac arrest, seizures). Although seizure activity and respiratory depression caused by LA overdose are potentially life threatening, these events can be readily treated with antiepileptic medications and controlled artificial ventilation, respectively. Of equal or greater importance, few options (e.g., ACLS) currently exist for treatment of cardiac toxicity caused by intravascular injection. For these reasons, an agent or technique allowing rapid, efficacious treatment of the cardiac effects of LA toxicity would be useful. The objectives of this grant are to generate the knowledge necessary to create agents specifically designed to treat patients suffering from the toxic effects of LAs. Recent advances in particle science engineering now afford new and exciting opportunities to develop highly effective therapeutic strategies aimed at successfully treating drug poisonings. Specifically, the recent advent of nanotechnology with its tremendous potential to solve major biomedical problems now offers unparalleled opportunities to solve the problem of LA toxicity. Four types of biocompatible and biodegradable nanoparticles (NPs) with 10-100 nm diameter will be synthesized by colleagues in the NSF Engineering Research Center for Particle Science and Technology for detoxification of LAs. These NPs will rely on absorption (microemulsions), adsorption (electron acceptor), or both mechanisms (2 types of "smart" microemulsions) to reduce the free concentration of LA in various media and decrease the biological effects of LA in tissues and intact organisms. The NPs will be studied to 1) detail the physicochemical characterization of the NP-LA interaction (Objective A), and 2) determine whether the cardiotoxic effects of LAs can be attenuated by NPs in biological systems (Objective B). This highly multidisciplinary project spanning organic chemistry, engineering, and medicine contains two objectives and three specific aims: Specific Aim #1: Determine the extraction efficiency of NPs to remove LAs from simple (normal saline) and complex (human plasma and blood) media. Optimize LA extraction efficiency of the various NPs. Specific Aim #2: Determine the molecular mechanisms whereby the different types of NPs can efficiently extract LAs. Specific Aim #3: Determine the effectiveness of nanoparticles to attenuate or reverse the cardiotoxic effects of LAs at three functional levels: 1) single cell (ventricular myocytes), 2) tissue (isolated hearts), and 3) intact rat (closed chest).

Ding, Ye   New York State Dept Of Health   Rational Design Tools For Antisense Nucleic Acids   GM068726   4 years   NIGMS

Abstract: The long term goal of this project is to develop novel algorithms and methods for improved prediction of RNA higher order structures, and for the rational and efficient design of antisense nucleic acids. Antisense oligonucleotides, trans-cleaving ribozymes and short interfering RNAs have emerged as increasingly important RNA-targeting tools for achieving efficient gene down-regulation. They are essential for high throughput functional studies of genes and gene products in humans, model organisms and infectious pathogens, as well as for the identification and validation of new therapeutic targets and agents against human diseases. To be effective, these antisense nucleic acid molecules require good target accessibility, which is primarily determined by the secondary structure of the target RNA. The secondary structures of mRNAs and viral RNAs are generally unknown, and are difficult to elucidate by experimental means. Therefore, computational methods could be valuable for the RNA structural determination. However, conventional RNA folding algorithms have not adequately addressed either the issue of uncertainty in the prediction or the issue of potential alternative structures for long-chain RNAs. Recently, a novel statistical sampling approach to RNA secondary structure prediction has presented a satisfying solution to these longstanding problems. This new method has been shown to offer important improvements for the prediction of messenger RNA structures and effective antisense targets, when compared to conventional methods. The objective of the present application is to develop algorithms and a methodology for the rational and efficient design of trans-cleaving ribozymes. This will be achieved by taking advantage of the statistical sampling method for target accessibility prediction and ribozyme design (Aim 1), by experimentally testing the computationally designed ribozymes both in vitro and in vivo, and to further improve the design methodology through statistical analysis and modeling of the experimental data (Aim 2). Finally, a software module incorporating the ribozyme design tools will be developed and made available to the scientific community through a Web server (Aim 3). Improved algorithms for RNA higher order structure prediction and more effective methods for the engineering of antisense nucleic acids are expected to result from this project. In the post-genomic era, the availability of the software and the Web server will substantially facilitate applications of antisense nucleic acids in high throughput functional genomics.

Disbrow, Elizabeth A   University Of California Davis   Linking Functional Imaging, Neurophysiology & Anatomy   NS044590   5 years   NINDS

Abstract: Brain imaging methods such as functional magnetic resonance imaging (fMRI), magnetic source imaging (MSI) and diffusion tensor imaging (DTI) are rapidly evolving as essential tools for assaying normal and abnormal brain function. The overall goal of this research is to enhance our understanding of the relationship between the signals measured using these imaging techniques and the underlying neural activity. We propose to conduct a series of experiments in anesthetized macaque monkeys to examine the correlation between functional brain imaging signals, specifically the BOLD signals of fMRI, the modeled current sources of MSI, and the imaging of white-matter tracts with DTI, with "gold standard" single and multi-unit electrophysiological recordings, and neuroanatomical tracing techniques. The specific aims are 1) To measure the stimulus evoked changes in magnitude, location and timing of functional brain imaging signals and relate them to changes in underlying neural activity, 2) To correlate non-invasive anatomic connectivity measures derived from tractography of DTI with connectivity derived using neuroanatomical techniques, and 3) To compare measures of functional connectivity based on the covariance of fMRI and MSI time-series with anatomic connectivity derived from DTI and neuroanatomic studies. These experiments represent a unique collaborative effort to combine several techniques in the same animal to generate a better understanding of the ability of modem imaging techniques to track changes in the nervous system under varying stimulus conditions and to uncover the circuitry necessary for complex sensory abilities. Our efforts are among the first to bridge the gap between imaging, neurophysiology and anatomy, an essential step in relating the wealth of electrophysiological recording data from macaque monkeys to the human cortex, and in understanding complex functions such as the sensory integration necessary for cognitive processes like object recognition and language.

Dong, Cheng	Pennsylvania State University-Univ Park	Neutrophil-Mediated Melanoma Cell Adhesion And Migration	CA097306	4 years	NCI

Abstract: The events that mediate the initial steps in melanoma extravasation, adhesion of melanoma cancer cells to the endothelium within the dynamic circulation, are not well defined. Studies from our group, as well as others, have suggested that melanoma cell lines inefficiently bind endothelial cells underflow conditions and may require assistance for adhesion. We propose that neutrophils (PMNs), through direct cell-cell interaetions and the elaboration of soluble factors, directly participate in this process by inducing tumor cell expression of chemokines and cellular adhesion molecules. These tumor-derived factors contribute to a microenvironment that enhances the ability of melanoma cells to adhere to endothelium within the circulation. Preliminary data are presented that support this model including results that show PMNs aggregate with melanoma cells, PMNs stimulate IL-8 production in melanoma cell lines and, most importantly, PMNs facilitate melanoma cell adhesion to endothelial molecules under flow conditions and subsequent extravasation. We aim to extend these studies by (1) developing an experimental system that will permit in vitro investigations of melanoma cell adhesion to endothelium and subsequent transmigration, especially in the presence of PMNs under flow conditions; (2) determining what cytokines and chemokines are produced by melanoma cells following PMN induction and their role in mediating tumor cell adhesion and migration; and (3) characterizing the signal transduction cascades and transcriptional activators in melanoma cells induced by PMNs that facilitate melanoma adhesion and migration under flow conditions. We wilI also examine the effect of endothelial activation on regulating melanoma cell adhesion and migration, especially under the influence of PMNs. Therefore, these studies will provide a better understanding of the potential accessory role of PMNs in melanoma cell binding and insight into general mechanisms of tumor cell adhesion under dynamic flow conditions that mimic a blood capillary. Furthermore, since adhesion is a critical step in tumor metastasis these studies may identify novel therapeutic targets against invasive melanomas.

Douglas, Gordon C	University Of California Davis	Flow Effects On Endothelial/Trophoblast Interaction	HL068035	4 years	NHLBI

Abstract: In humans and higher primates, fetal trophoblasts gain access to the lumens of dilated uterine capillaries and migrate along endothelium against the flow of blood eventually remodeling spiral arteries. Our general hypothesis is that migration of trophoblasts within uterine blood vessels is regulated by blood flow-derived shear stress. New data now show that trophoblasts migrate against flow when cultured on top of confluent uterine endothelial cells. To account for these observations, two, not mutually exclusive, mechanisms are proposed. First, we suggest that shear stress causes an asymmetric distribution of immobilized chemokines or adhesion molecules on endothelial cells that generates a haptotactic gradient directing trophoblast migration against flow. Second, we suggest that interaction of trophoblasts with immobilized chemokines and/or adhesion molecules on endothelial cells results in trophoblast activation causing trophoblasts to respond to shear stress by migrating against flow. Four aims will allow us to distinguish these mechanisms and characterize the features that define the shear stress-dependent migratory phenotype. First we will use videomicroscopy to characterize the effect of different levels of shear stress on the directional migration of trophoblasts on uterine endothelial cells. Aim 2 uses confocal microscopy to study the role of chemokines and endothelial adhesion molecules in shear stress-mediated trophoblast migration. We will also use migration checkerboard assays to determine whether chemokine- or adhesion molecule-induced trophoblast migration is haptotactic, chemotactic or chemokinetic. Aim 3 determines the effect of shear stress and trophoblast-endothelial interaction on induction of a migratory trophoblast phenotype. We will use confocal microscopy to characterize the leading edge/trailing edge distribution of CCR5, beta1 integrin, and cytoskeletal elements in trophoblasts exposed to different levels of shear stress in the presence of endothelial cells. Expression of these proteins will be quantiated by laser scanning cytometry, immunoblotting, and quantitative RT-PCR analysis. Function-blocking antibodies will identify the role of trophoblast adhesion molecules and chemokine receptors in flow-induced migration on endothelium. Aim 4 will use quantitative laser scanning cytometry to examine the expression of chemokine receptors, chemokines, and adhesion molecules in intravascular trophoblasts and endothelial cells in serial sections of uterine tissue.

Drueckhammer, Dale G State University New York Stony Brook Fluorescence-Based Glucose Sensors DK059568 3 years NIDDK

Abstract: The goal of this project is to develop a fluorescence-based sensor for glucose that may be used for the continuous monitoring of blood glucose levels in diabetes patients. This project is based on previous observations that the fluorescence of an arylboronic acid may be altered upon formation of a complex with glucose. While the change in fluorescence can be used to determine glucose concentration in solution, it is not specific for glucose as other sugars and related compounds can also induce the fluorescence change. The goal of this project is to develop new methods for building in high selectivity for complex formation with glucose in systems chosen such that the extent of glucose binding can be conveniently determined by fluorescence measurements. This will provide the basis for a practical sensor for the measurement of glucose concentration in the presence of a variety of sugars and other potential interfering substances. The design of specific glucose receptors will be based on computer-aided design of molecular structures that permit the precise positioning of multiple functional groups that will bind to different parts of a glucose molecule. Structures designed by this approach will be synthesized and the binding of glucose and accompanying fluorescence changes will be studied. Initial structures will be further modified to tune the sensor to physiological glucose concentration and other practical issues in glucose sensor development will be addressed.

Ewing, James R Case Western Reserve Univ-Henry Ford Hsc MRI Measure Of Blood Brain Barrier Permeability HL070023 4 years NHLBI

Abstract: The blood-brain barrier is a unique feature of cerebral vasculature, characterized at the capillary level by tight intercellular junctions devoid of fenestrae, and isolating the brain from the rest of the body. In many cerebral pathologies, a breakdown of the blood-brain barrier is a characteristic of the disease; examples of such pathologies are reperfused cerebral ischemic infarction and cerebral tumor. We propose to develop magnetic resonance imaging (MRI) techniques that assess the permeability of the cerebral vascular bed for a wide range of compounds. From small to large these are: Gd-DTPA, gadomer-17, albumin-complexed Gd, and ultrasmall particles of iron oxide (USPIO's). These compounds will be used in reperfused cerebral ischemic infarction and cerebral tumor. We have developed techniques to measure blood-brain barrier (BBB) permeability to MRI contrast agents in rat models of reperfused cerebral ischemic infarction and cerebral tumor. We have developed, refined, and confirmed in preliminary studies, theories for measuring BBB permeability to water and to magnetic resonance contrast agents. We propose to further develop MRI techniques and analyses that determine the transfer constant and permeability-surface area product (PS product) of MRI contrast agents in rat models of reperfused cerebral ischemic infarction and cerebral tumor. Additionally, we will investigate MRI methods to estimate the extracellular, extravascular volume fraction of the cerebral tissue, and the relative cerebral blood volume (rCBV) of the cerebral vasculature. We will demonstrate these measures in 9L tumors in Fischer 344 rats, and in reperfused ischemic infarction in Wistar rats.

Folch, Albert University Of Washington A Nanofluidic Device For Synaptogenesis Studies EB001474 5 years NIBIB

Abstract: A major goal in neuroscience is to understand the formation and development of synapses, the tiny membrane specializations that enable nerve cells to communicate with each other. The sequence of molecular signals leading to synapse formation ("synaptogenesis") is qualitatively well known for the more accessible neuromuscular synapse. It is well established that, immediately after contacting the muscle cell, the nerve terminal secretes agrin to induce the clustering of acetylcholine (ACh) receptors at the postsynaptic site. After a cascade of events, the nerve is able to depolarize the muscle cell by releasing pulses of ACh. However, very little is known of the quantities (concentration, duration, onset, etc.) of the various neurochemical signals involved in synaptogenesis. Importantly, all except for one of the axons innervating a given myotube at birth retract after a period of a week or so according to a synaptic competition process that remains, for lack of quantitative methods, poorly understood. Such quantitative description is lacking because present experimental setups for the study of the neuromuscular junction do not allow for a precise control over the many variables involved in synaptogenesis. Therefore, we propose a quantitative approach based on substituting the presynaptic neuron by an artificial mimic, a nanofluidic device that will stimulate the muscle cell in a physiologically relevant way. We hypothesize that the focal delivery of synaptogenic factors will recruit the synaptic machinery to the stimulated area of the membrane. The device will consist of a set of microfluidic channels buried underneath the cell culture surface and that will "communicate" with the cells through nanoholes. The cells will be sealed to the holes by applying suction to the microchannels. Unlike present experimental setups, our device will allow us to 1) confine the delivery of agrin/ACh to a submicron-diameter area of the cell membrane (as occurs in vivo); 2) interrogate the same area of the membrane with different factors sequentially; 3) stimulate several locations of the same cell simultaneously (with the same or dissimilar stimuli); and 4) experiment with high throughput (i.e. investigate large numbers of cells and stimulation conditions simultaneously). The proposed device has broad applicability to cell culture studies requiring nanometer-scale, focal exposure of the cells to a soluble factor.

Franzen, Stefan   North Carolina State University Raleigh   Multifunctional Nanoparticles For Intracellular Delivery CA098194   4 years   NCI

Abstract: This proposal describes the synthesis of a general class of nanoparticle delivery vectors based upon hybrid biomolecule-gold nanoparticle complexes. The vectors are designed to transport therapeutic oligonucleotides across cell membranes to target cancer cell nuclei. Therapeutic oligonucleotides are synthetic single stranded nucleic acid molecules that are resistant to digestion by nucleases. A multifunctional approach has been developed which combines cell-specific recognition ligands, endosomal escape peptides, nuclear localization signals and controlled release of therapeutic oligonucleotides to cells. The ability to target biomolecule-nanoparticle complexes to specific cell types, through a relatively simple attachment of cell-specific ligands or peptides, provides the potential for diagnosis and treatment of cancer. A quantitative test for nuclear delivery of an oligonucleotide drug to the nuclei of HeLa cells is proposed as an assay for the efficiency of delivery of a therapeutic agent. The long term goal of this research is to develop a non-viral vector capable of delivering therapeutic oligonucleotides to cancer cells in vivo. Achieving this long term goal requires the following steps. 1. Formulate stable nanoparticle-bioconjugates that are resistant to aggregation and chemical exchange in biological fluids and cells. 2. Develop microscopy techniques to monitor nanoparticle trajectories and quantitate localization in cells. 3. identify the best combination of peptides for performing the functions of endocytosis, endosomal escape and nuclear uptake. 4. Characterize fundamental interactions of protein-peptide conjugates with nanoparticles including their susceptibility to exchange or replacement. 5. Develop strategies for covalent attachment of proteins and oligonucleotides to nanoparticles. 6. Determine of the efficiency of regulated in vitro protein expression using a well-characterized model system.

Garcia, Andres J   Georgia Institute Of Technology   Genetic.Engin.Cells/Scaffolds For Bone Tissue Engineering EB003364   4 years   NIBIB

Abstract: Tissue-engineered constructs, consisting of cells and 3-D scaffolds, have emerged as promising alternatives to biological and synthetic grafting materials for the repair of non-healing bone defects. However, host tissue-construct interactions, loss of osteoblastic phenotype under culture conditions, and limited supply of committed osteoprogenitor cells that will differentiate into osteoblasts restrict this approach. The objective of this application is to integrate cells genetically modified to express the osteoblast transcription factor Runx2/Cbfal into 3-D scaffolds to create hybrid constructs that promote matrix mineralization in vitro and in vivo. Our central hypothesis is that tissue-engineered constructs containing Runx2-expressing cells will significantly promote matrix mineralization in vitro and in vivo and enhance the healing of critical-size defects compared to constructs containing unmodified cells or empty scaffolds. The rationale for this work is that it will establish a genetic engineering strategy to overcome cell sourcing limitations associated with the application of tissue engineering to treat bone defects. Aim 1: Analyze the effects of Runx2 expression on gene/protein expression and matrix mineralization by cells cultured in 3-D polymeric scaffolds in vitro. We hypothesize that controlled expression of Runx2 enhances gene/protein expression and promotes matrix mineralization in 3-D constructs compared to unmodified cells. Aim 2: Examine the extent of bone-specific protein expression and mineralization in Runx2-engineered cells/scaffold constructs implanted into a subcutaneous site. We hypothesize that tissue-engineered constructs containing Runx2-modified cells exhibit enhanced in vivo ectopic bone formation compared to constructs containing unmodified cells. Aim 3: Evaluate the ability of tissue-engineered constructs containing Runx2-modified cells to repair critical-size bone defects. We will test the hypothesis that constructs containing Runx2-expressing cells promote in vivo bone formation and enhance repair of non-healing calvaria defects in syngeneic rats compared to cell-free scaffolds and constructs containing unmodified cells. This work is expected to yield the following outcomes: (1) establish the extent to which Runx2 expression enhances in vitro matrix mineralization and identify experimental parameters (scaffold pore size, dynamic culture conditions) that enhance mineralization in tissue-engineered constructs; (2) assess the ability of these constructs to form bone tissue in a non-osseous environment and provide information on the overall host response to these constructs; and (3) establish the potential of this genetic/tissue engineering approach to repair non-healing bone defects. Finally, these in vivo studies will also provide a solid foundation for validating the in vitro and subcutaneous models as surrogates for in vivo responses in an osseous environment. Collectively, these outcomes will validate this genetic engineering strategy for addressing cell sourcing issues in tissue engineering applications and establish the effectiveness of tissue engineering approaches to repair non-healing bone defects.

Geng, Lei   University Of Iowa   Spectrally-Resolved Fluorescence Correlation Imaging   CA100741   5 years   NCI

Abstract: Fluorescence imaging offers excellent sensitivity that is unparalleled by any other optical imaging techniques. It has thus been one of the most powerful tools in biomedical applications. The drawback of fluorescence intensity imaging, however, is the low information content and a lack of chemical specificity. This project aims to develop a novel technique, spectrally-resolved fluorescence correlation spectroscopy (FCS), for biomedical imaging. In this new imaging method, fluorescence response to an external field is collected at a range of wavelengths. Time correlation function is evaluated between wavelengths to yield a two-dimensional fluorescence correlation spectrum for each spatial location in an image. By coupling spectral and time resolution, spectrally-resolved FCS greatly enhances the information content and thus provides (1) high contrast in diagnostic imaging and (2) detailed information on the physicochemical environments of the fluorescent probe. The high contrast of spectrally-resolved fluorescence correlation imaging is applied to a model system of cancer diagnosis. Cancers are the second leading cause of death in the United States, accounting for 25% of the total fatalities. The key to timely treatment of cancers and improved survival rate is early diagnosis. We plan to use the new method to examine tissue samples in various pathological conditions, from healthy, hyperplastic, adenomatous to adenocarcenomatous. Spectroscopic measurements of tissue samples collected in surgery and biopsy procedures are conducted in vitro. Spectral libraries are established for normal and cancerous tissue through fiber optical examination of tissue samples. Computer programs are written for data treatment and statistical analysis of the library spectra. Various decision parameters are explored to establish the best diagnostic criteria. Coupling both spectral and temporal resolution of fluorescence, the new method provides significantly improved contrast between the normal and cancerous tissues compared to the existing method of steady-state fluorescence spectroscopy. With the enhanced contrast, spectrally-resolved fluorescence correlation imaging holds excellent potential in noninvasive diagnosis of cancers.

Gluckman, Bruce J   George Mason University   Electric Field As Novel Neuronal Interface   EB001507   4 years   NIBIB

Abstract: An applied electric field can be used to modulate a neuron' s activity. Although the advantage of employing fields for implementation in the design of neural prosthetics was recognized from invertebrate research, the field lay fallow for nearly a decade. Over the past five years, we have moved this field forward significantly. In recent papers, we have demonstrated that a DC electric field could be used to suppress or enhance epileptiform activity in hippocampal slices (Gluckman, et al., 1996a), and, when applied adaptively, could turn off seizures indefinitely (Gluckman, et al., 2001). One advantage of using electric fields to interact with neuronal networks is that, if properly instrumented, it can be done simultaneously with ongoing measurement of neuronal activity. Therefore, feedback can be easily implemented. But, one of the reasons electric fields have not been pursued is that readily available measurement and stimulation electronics are not easily adaptable for use with electric field stimulation. In addition, until recently, biocompatible electrode materials with sufficient charge passing capacity to produce sustained electric fields were not available. The aims of this project are to translate our existing seizure control techniques for chronic in vivo animal use. This will require design of instrumentation for simultaneously stimulating with electric fields and recording neuronal activity in intact brain, to establish safety limits for biocompatible electrodes under electric field stimulation, and to develop and test feedback seizure control algorithms. The instrumentation and methods developed will be prototypes of a novel neuronal interface based on electric field stimulation. Such an interface would lay the groundwork for a new generation of medical devices to treat dynamical diseases of the brain such as epilepsy, to provide an interface for neuronal prosthetics, as well as provide an arsenal of new tools for probing the complex dynamics of neuronal systems.

Grainger, David W | Colorado State University | DNA Microarray Surface Analysis To Optimize Detection | EB001473 | 4 years | NIBIB

Abstract: Microarray-based information, now routine in most medical research communities, pervades most aspects of diagnostics, sensing, biotechnology, oncology and pathology. Detection limits and selectivities for DNA targets are far below theoretical performance limits, but very little information is reported or known about the chemical, physical or biological fate of full length DNA, cDNA or oligo-DNA probes immobilized on any of the diverse set of microarray surfaces. How immobilized DNA surface disposition influences subsequent hybridization efficiency, and reliability of array data interpretation and assay quantification is unknown. Quantitative interpretation of DNA microarray signal intensity is currently very difficult since factors influencing DNA probe-target interactions at microarray surfaces have not been analyzed with high-resolution surface analytical methods often applied to other biomedical surface problems. Our hypothesis is that DNA microarray target hybridization efficiency and diagnostic target detection limits in biomedical samples are correlated directly with the orientation, density, and immobilization efficiency of probe DNA on microarray surfaces. To investigate this hypothesis, we propose the following Specific Aims: 1. Establish a quantitative understanding of the correlation between immobilized probe DNA density on microarray surfaces and target hybridization efficiency in biological samples using radiometric 32P-DNA assay and optical imaging on several surface chemistry platforms and assess reliability and reproducibility issues in these strategies; 2. Develop reliable, quantitative methods for high-resolution surface analysis of DNA density and orientational populations on ss-cDNA and hybridized ds-DNA on arraying surfaces using modem biomedically relevant methods (XPS, ToF-SIMS and optical anisotropy of immobilized DNA). The overall objective is to correlate high-resolution surface analytical data on DNA arrays with radiometric measurements to establish a non-radiometric 'standard curve' to assess DNA immobilization on microarray surfaces more conveniently and accurately. Moreover, orientational information on immobilized DNA using a combination of innovative spectroscopy methods will be correlated to immobilized DNA density and hybridization efficiency in array formats. All methods will converge to produce a fundamental understanding of microarray surface & hybridization performance limitations currently not available.

Guilak, Farshid | Duke University | Biomechanical Factors In Rheumatoid Arthritis | AR048182 | 3 years | NIAMS

Abstract: Rheumatoid arthritis is a chronic arthropathy characterized by inflammation, proliferation and destruction of the articular cartilage. Although historically cartilage has been considered to be an "innocent bystander" of the disease, recent evidence suggests that the degradation of cartilage in arthritis involves an imbalance of the anabolic and catabolic activities of the articular chondrocytes, secondary to synovitis and joint inflammation. Chondrocyte metabolic activity is strongly influenced by soluble mediators (e.g., cytokines) and biophysical factors (e.g., mechanical stress). In particular, biomechanical factors may play an important role in the onset and progression of degenerative arthritis secondary to joint inflammation in rheumatoid arthritis. However, the sequence of biomechanical and biochemical processes regulating these events in vivo is still unclear. The primary hypothesis of this study is that, in rheumatoid arthritis, a loss of cartilage biomechanical function and the presence of inflammatory cytokines alters the metabolic response of chondrocytes to mechanical stress. Aim 1 of this project is to measure the mechanical properties of the cartilage extracellular and pericellular matrices in RA, and to incorporate this data in a theoretical model of the micromechanical environment of the cell. In Aim 2, we will determine the role of stress magnitude in the stimulation of nitric oxide and prostaglandin E2 production by chondrocytes, and determine the influence of these inflammatory mediators on matrix turnover. In Aim 3, we will determine whether mechanical stress has an additive or antagonistic effect on with certain inflammatory cytokines (interleukin 1, tumor necrosis factor alpha, and interleukin 17) in controlling the PGE2 synthesis and matrix metabolism. Currently, there is little information on the biomechanical changes in articular cartilage with RA. Understanding the biomechanical and molecular mechanisms of chondrocyte response to physiologic loading in an inflammatory environment may enable new therapies that are specific to the stage of the disease. As many pharmacologic therapies for RA are focusing on the NOS2 and COX2 pathways, investigation of the interaction of physical therapies with these pathways will hopefully lead to more safe and effective treatments for RA.

Hashsham, Syed   Michigan State University   Flexible Biochip For Highly Parallel Microbial Detection   RR018625   3 years   NCRR

Abstract: We propose to develop a biochip for parallel detection of thousands of microorganisms. It will serve as a genetic screen for the parallel detection, identification, and quantification of up to 10,000 unique microorganisms or gene targets and can be extended to include up to 30,000 targets in the near future. The need for such a comprehensive and broad-range screening tool has been recognized for years in many areas including diagnostics, air, water, food, animal, and plant safety, and bioprocess monitoring. However, its development was hampered by the ability to synthesize and test thousands of possible probes rapidly and economically. This problem is solved now because of the invention of a highly flexible and low-cost in-situ oligonucleotide biochip synthesis platform by the University of Michigan and Xeotron. It can produce biochips overnight without costing a fortune. It is highly flexible with respect to probe content which is software controlled. It uses traditional DNA synthesis chemistry modified to respond to light and thousands of microfluidic reactor array etched on silicon surface. This proposal combines the expertise in bioinformatics, phylogenetics, environmental engineering, and data interpretation for mixed microbial systems available at the Center for Microbial Ecology (CME) at Michigan State University (MSU) with the flexible biochip fabrication technology described above. Probes for the biochip will be designed and validated by researchers at CME, which is a world leader in developing new methodologies to track and identify microbes in complex microbial systems. Synthesis of the biochip will be carried out by UM and Xeotron. Xeotron currently has a $9M phase one venture capital and is fully geared to produce any chip developed on this platform. Ability to detect all microorganisms of interest in a given matrix together will significantly improve the management of health concerns related to microorganisms.

Haugh, Jason M   North Carolina State University Raleigh   Molecular Crosstalk In Intracellular Signaling Networks   GM067739   4 years   NIGMS

Abstract: The most widely studied signaling system in cell physiology is arguably the regulatory switch governing proliferation and programmed cell death. Not surprisingly, mitogens such as platelet-derived growth factor (PDGF) trigger proliferation, through the Ras/Erk pathway, as well as protection from cell death, through the phosphoinositide 3-kinase (PI3K)/Akt pathway. Confounding the analysis of these pathways are the numerous crosstalk interactions between them, which suggest that cell life and death are co-regulated. A quantitative understanding of crosstalk in signaling networks is thought to be a major hurdle in the design of molecular therapeutics targeting intracellular signaling proteins, with implications for cancer, wound healing, and immune cell regulation. Employing PDGF-stimulated signaling in NIH 3T3 fibroblasts as a model system, the magnitudes and kinetics of Ras/Erk and PI3K/Akt signaling will be manipulated through specific genetic and pharmacological interventions, and crosstalk will be assessed by measuring the sensitivities of other intermediates to those changes for various levels of receptor stimulation. This general strategy is termed crosstalk titration. Central to these efforts are quantitative, high-throughput cell biochemical assays for the levels of PDGF beta-receptor phosphorylation, Ras-GTP, Akt kinase activity, and Erk kinase activity. With extensive signaling data for various PDGF concentrations and intracellular manipulations, a mathematical model of the signaling network will be formulated to predict the outcomes of more complex intervention strategies. Finally, for conditions found to perturb the signaling network, the corresponding effects on cell proliferation and survival will be assessed.

Ismagilov, Rustem F   University Of Chicago   New Microfluidic Technology As Research Tool For Biology   EB001903   5 years   NIBIB

Abstract: Research in genomics and proteomics is constantly identifying -- by qualitative screening -- thousands of new molecules of proteins, DNA and RNA critical to health or responsible for a disease. Detailed understanding of the structure and function of these molecules is of fundamental importance to biology and medicine, but it is difficult to achieve by traditional methods, which are labor intensive and consume large amounts of samples. Microfluidics allows manipulations and monitoring of minute volumes of solutions, and is attractive as the key technology for overcoming limitations of traditional methods. However, microfluidic devices have not yet found widespread applications as research tools. An intense research effort is directed towards solving three problems of microfluidics: i) large dispersion of solution along the channels increases consumption of reagents and also makes long (minutes to days) time scales difficult to access; ii) slow mixing of solutions makes very short (tens of milliseconds and below) time scales inaccessible; mixing approaches that rely on turbulence prohibitively increase the sample consumption; iii) chemistry of internal surfaces of devices is important and has to be controlled. This multi-disciplinary program will begin by developing a new microfluidic technology that is universal -- it will be useful for quantitative, high-throughput experiments on time scales ranging from tens of microseconds to days. Dispersion will be eliminated by localizing reagents inside aqueous droplets encapsulated by an immiscible fluid. Mixing inside the droplets will be accelerated by using chaotic advection, rather than turbulence. Surface chemistry to which solutions are exposed will be controlled by careful choice of surfactants. This program will then demonstrate the utility of this technology for biomolecular functional and structural studies. It will be used as the basis for unique kinetics systems suitable for measurements of dynamics on time scales from tens of microseconds to seconds. This kinetics system will be applied to measurements of fast enzyme kinetics and early events in RNA folding. This technology will also be used for protein crystallization to control both short time scale events such as nucleation, and to control long time scale events such as growth. Finally, the program will develop methods for making these technologies user-friendly and implement them in laboratories of our collaborators. This technology will improve health by enabling basic research. It will not only enable measurements and experiments that are impossible to do today; it will also make these measurements rapid, economical, and accessible to a wide community of researchers in biology, biophysics, and bioengineering.

Kelso, David M   Northwestern University   Spectral Imaging System For Encoded Particle Arrays   EB001418   3 years   NIBIB

Abstract: The goal of this research is to develop an automated multispectral imaging system to read protein arrays based on quantum dot encoded microparticles. Quantum dots, QDs, have recently been embedded in microparticles to generate large numbers of unique fluorescent codes. QDs are an ideal means of fluorescently encoding microparticles since they can be synthesized with emission peaks across the entire visible spectrum, yet they all have a common near-UV excitation wavelength. Tunable filters have recently been developed with the spectral and spatial resolutions needed to image 1 um diameter particles. The combination of QD encoded particles and a tunable filter makes possible novel approaches to multiplexing which could greatly increase the number of identifiable codes, and at the same time, simplify particles synthesis. Our approach is to create a binary coding scheme where each species of QD is either present or absent from the particle. By decreasing the separation between emission peaks to approximately 10 nm, theoretically it will be possible to generate more than 10[6] unique codes. Specifically, we propose to: 1) Install a tunable filter in our automated imaging system to enable collection of spectral data, 2) Write software to automate spectral image acquisition, 3) Develop algorithms for particle segmentation and classification. 4) Determine optimum ranges for QD intensities and separation of emission maxima, 5) Extend acquisition, segmentation, and classification algorithms to sedimented particles, and 6) Demonstrate immobilized and sedimented encoded particle technologies with model assay systems and evaluate assay performance. Development and testing will be greatly facilitated by incorporating the QD-coded particles into our immobilized particle arrays. The QD-encoded particles will be arrayed on hydrogel-coated slides to provide training sets with well-controlled particle densities on optically flat surfaces. They will be used to evaluate the filter performance and develop image processing algorithms. In the latter phases of our proposed research, we plan on extending the method to particles sedimented in microtiter wells and demonstrate the feasibility of detecting proteins bound to QD-encoded particles.

Kelso, David M   Focus/MRL, Inc.-MRL Pharmaceutical Srvs   Diagnostic Microarray For West Nile Virus Infections   AI056647   1 years   NIAID

Abstract: Rapid, accurate detection of West Nile virus (WNV) infections can alert public health officials to increase preventative measures and control outbreaks. By applying our novel immobilized particle microarray technology to the detection of antibodies to WNV, and making it compatible with 384-well microplates, chemical kinetic theory predicts we can: 1) eliminate processing steps by performing both assays in a single array and replacing the enzyme-substrate reporter chemistry with a fluorescent label; 2) minimize reagent usage by reducing the capture surface area several orders of magnitude; 3) improve precision by increasing the fractional occupancy of each capture site and minimizing effects of capture surface area and reaction volumes; 4) decrease turnaround times by removing diffusion limitations and shortening processing steps; and 5) demonstrate sensitivity and specificity equivalent to current tests. The technology can also be applied to a number of other viral agents which are currently monitored by the CDC, or may be employed in bioterrorist attacks. There should be significant commercial potential for diagnostic products resulting from this research because they will provide higher throughput, better precision, and lower costs.

Krassowska, Wanda   Duke University   Stability Of Cardiac Response To Rapid Pacing   HL072831   4 years   NHLBI

Abstract: In this multidisciplinary project, researchers from Biomedical Engineering, Pediatric Cardiology, Physics, and Mathematics will combine theoretical and experimental approaches to investigate the stability of cardiac response under rapid pacing. As the pacing rate increases, cardiac muscle exhibits beat-to-beat changes in the action potential duration (APD alternans). APD alternans and its clinical manifestation, T-wave alternans, are associated with increased vulnerability for arrhythmias. Thus, increased understanding of the rhythm stability coming from the proposed research will lead to the development of new techniques for detection of the precursors of arrhythmias and for identifying patients at risk for fibrillation and tachycardias. Specific Aims are: (1) Develop a new, more robust experimental protocol for determining stability of the cardiac response pattern. (2) Investigate whether steady-state alternans occurring in spatially extended, homogeneous tissue is always discordant (i.e., APD oscillations in some regions of the tissue are out of phase with the oscillations at the pacing site). (3) Construct an optical fiber-based transmural mapping system that allows mapping action potentials in three dimensions. The research will start with mathematical analysis that uses idealized models of membrane kinetics. Analytical results will be tested through computer simulations, first involving idealized membrane models under space clamp conditions, then progressing to physiologically accurate membrane models in up to three spatial dimensions. Some models will also take into account transmural APD heterogeneity. Concurrently, analytical results will be tested experimentally. Initial tests will use in-vitro preparations of bullfrog ventricle, which are relatively, and progress to in-vitro wedge preparations of rabbit ventricle, which exhibit transmural APD heterogeneity and anisotropy typical for mammalian hearts. The three-way comparisons between results from theory, computer simulations, and experimental studies will allow us to refine and, if needed, expand the mathematical theory and computer models, with each iteration leading to more complete understanding of cardiac dynamics.

Kuiken, Todd A   Rehabilitation Institute Research Corp   EMG Propagation In Planar Muscles For Prosthesis Control   HD043137   5 years   NICHD

Abstract: Currently upper-limb amputees can only operate a single degree-of-freedom at a time with myoelectric prostheses. This is very inadequate, especially for high-levels of amputation such as shoulder disarticulation(SD) where multiple functions need to be controlled. We postulate that the residual brachial plexus nerves in a SD amputee can be grafted onto separate regions of the pectoralis major (pmajor) muscle and that these nerve-muscle grafts could provide additional myoelectric control signals that are physiologically related to the functions they would be controlling in the prosthesis. This would allow simultaneous control of multiple degrees-of-freedom with a more natural feel. The technique has great potential for improving the control of myoelectric SD prostheses. The key to success with this technique will be the ability to record independent surface EMG signals from each of the nerve-muscle grafts. In order to study EMG signal independence in the chest, a series of finite element (FE) computer models of EMG signal propagation in the chest will be developed and validated with experimental data. Using FE analysis, it is possible to simulate surface EMG signals under a range of different conditions. Effects such as muscle anatomy, biological tissue properties and recording electrode configuration will be investigated in a manner not possible using experimental methods. First, FE analysis will be used to investigate the relationship between surface EMG signal independence and the geometry of the active muscle, neighboring muscles and other tissues near the recording site. This will be accomplished with a series of generalized planar FE models. Next, finite element analysis will be used to determine the effect of anatomical manipulations for improving surface EMG signal independence including removal of fat, concentrating muscle tissue at recording sites and insulating muscles with a layer of fat. Finally, the subject-specific models will be used to simulate the nerve-muscle graft technique and test the feasibility of this novel approach. Anatomical manipulations to enhance surface EMG signal independence will also be tested with the subject-specific models.

Kuiken, Todd A   Rehabilitation Institute Research Corp   Nerve-Muscle Grafts In Amputees For Prosthesis Control   HD044798   3 years   NICHD

Abstract: Currently transhumeral amputees can only operate a single degree-of-freedom at a time with myoelectric prostheses. This is very inadequate since multiple functions need to be controlled. We believe that the residual brachial plexus nerves in a transhumeral amputee can be grafted onto separate regions of the biceps, triceps and brachialis muscles and that these nerve-muscle grafts could provide additional myoelectric control signals that are physiologically related to the functions they would be controlling in the prosthesis. This would allow simultaneous control of multiple degrees-of-freedom with a more natural feel. The technique has great potential for improving the control of transhumeral myoelectric prostheses. A series of experiments have been performed that indicate the concept is feasible with a high probability of success. Since the muscles will be 'hyper-reinnervated' by the donor nerve, good muscle recovery is expected. Extensive EMG modeling studies indicate that surface EMG signals from the different nerve-muscle grafts will be able to provide discreet and independent control signals allowing simultaneous operation of a terminal device, elbow and wrist rotator. We propose a small, carefully orchestrated clinical trial of the nerve-muscle graft technique in recent transhumeral amputees. Baseline testing will be done with a conventional myoelectric prosthesis to measure operational performance. With IRB approval, surgery will be performed to denervate the medial head of the biceps and brachialis muscles, then graft the median and distal radial nerves on to these muscles. Once the muscles are reinnervated, the patient will be fit with an appropriately modified myoelectric prosthesis and trained in its use. The patient will then be able to use the nerve grafts to control a myoelectric hand and the lateral head of the biceps and the triceps to control the powered elbow. Performance testing will be repeated with the experimental myoelectric system for comparison to conventional measures.

Kwon, Glen S   University Of Wisconsin Madison   Artificial Polymeric Lipoproteins As Drug Carriers   AI043346   4 years   NIAID

Abstract: The clinical role of many drugs currently used to fight opportunistic infections (OIs) and the impact of many potent drugs for OIs coming out of massive drug discovery programs have been hampered by poor watersolubility, high toxicity, and inadequate parenteral dosage forms despite encouraging results in preclinical and clinical testing. Current efforts to address these major bottlenecks in drug development fall in the realm of nanotechnology. In particular, polymeric micelles, nanoscopic supramolecular core-shell structures, have recently entered clinical trials for potent yet poorly water-soluble and toxic drugs, owing to safety, high drug loading, and improved pharmacokinetics. A unique aspect of polymeric micelles is the ability to adjust their chemical structures to fine-tune properties for drug delivery. Our results suggest that adjustments must be made with an individual drug or class of drugs in mind, and that easily made adjustments on poly(ethylene oxide)-block-poly(L-amino acid) (PEG-b-PLAA) micelles may enhance drug delivery. Our efforts focus on amphotericin B (AmB), the primary drug for opportunistic systemic fungal infections. These OIs are a major cause of morbidity among immunocompromised patients suffering from cancer or AIDS and organ transplant recipients. We believe that tailor-made PEG-b-PLAA micelles may increase the therapeutic index of AmB. Specifically, we hypothesize that beneficial changes in the pharmacokinetics of AmB, increased plasma halflife and reduced liver clearance, and changes in its self-aggregation state, owing to PEG-b-PLAA micelles may lower the drug's toxicity and increase its antifungal efficacy. In this context, we may adjust the structure of PEG-b-PLAA micelles to fine-tune the release kinetics of AmB and enhance its delivery. Specific Aims: (1) To study the pharmacokinetics (plasma profile, distribution in plasma, and tissue distribution) of AmB encapsulated by PEG-b-PLAA micelles in rodents. (2) To study the acute, renal and liver toxicity of AmB encapsulated in PEG-b-PLAA micelles in rodents. (3) To study the antifungal activity of AmB encapsulated in PEG-b-PLAA micelles in a neutropenic murine model of disseminated candidiasis. Comparisons will be made with a standard formulation of AmB and a liposomal AmB approved for refractory systemic fungal diseases. These proposed studies will provide insight into mechanisms behind the toxicity and antifungal activity of AmB and perhaps show that PEG-b-PLAA micelles increase the therapeutic index for the drug.

Lakowicz, Joseph R   University Of Maryland Balt Prof School   Metallic Surfaces And Particles In DNA Analysis   HG002655   3 years   NHGRI

Abstract: We propose to evaluate and develop the use of metallic particles to modify fluorescence as used in DNA analysis. Fluorescence measurements are usually performed in optically homogeneously media, providing little opportunity to modify fundamental spectral properties. In contrast, recent experiments from this laboratory have shown that proximity of fluorophores to conducting metallic surfaces can increase radiative decay rates, increase quantum yields, decrease lifetimes and improve photostability. We expect these effects to substantially increase the number of photons which can be emitted by a single fluorophore. We will use silver particles and coated metallic surfaces to: 1. Examine the effects of metallic surfaces on the intrinsic emission of DNA, bases and nucleotides. The metallic particles will include silver island films, annealed films, colloids, clusters of colloids and coated metallic surfaces. 2. Examine the effects of these metallic particles on extrinsic probes commonly used to label DNA, and to explore the use of low quantum yield probes which become fluorescent near metallic surfaces. 3. Determine the effects of metallic surfaces on the rates and maximum distances for fluorescence resonance energy transfer (RET). We expect RET near metallic surfaces to extend to hundreds of A, as compared with the usual upper limit near 60 Angstroms. 4. Evaluate the use of lanthanides and transition metal-ligand complexes on DNA arrays containing metallic surfaces. These longer lived probes are highly photostable and may become useful on DNA arrays with the increased emission rate expected near metallic particles. 5. Apply the knowledge gained from Specific Aims1-4 for use on DNA arrays designed for rapid identification of antibiotic, resistant bacteria and environmental pathogens such as C. dipthariae and V. cholerae. Most experiments will be performed using metallic silver, but gold will be examined with longer wavelength DNA probes. Both intensity and lifetime measurements will be used to separate increases in the radiative rates from other effects of the sample on the fluorophore. Both one-photon and multi-photon excitation will be used. We expect this project to determine how metallic particle effects on fluorescence can be used for new approaches to DNA analysis.

Lasky, Stephen R   Institute For Systems Biology   Fast, Flexible And Inexpensive Ink-Jet Array Printer   HG002931   3 years   NHGRI

Abstract: A new ink-jet oligonucleotide array synthesizer and several novel applications of this technology are being developed by the Institution for Systems Biology (ISB). When completed, specifications for construction, maintenance, and operation of this unique system will be released to the scientific community, satisfying the need for fast, flexible, and inexpensive synthesis of custom oligonucleotide array chips. Preliminary experiments comparing the results from ink-jet and pin-spotted microarrays for halobacterium, yeast, and murine T-cell receptor model systems reveal significant progress in the development of the ISB ink-jet arrayer. Despite considerable improvements, better consistency, density, and improved quality controls of in situ oligonucleotide synthesis are needed before this instrument is ready to be introduced into basic research laboratories. The most promising advantage of in situ ink-jet array synthesis over alternative methods is its speed and flexibility; new arrays can be designed and printed overnight without the need to catalog and array thousands of PCR gene products. We intend to take advantage of this flexibility to approach new challenges. In addition to gene expression analysis, we will adapt ink-jet chips to study the effects of gene knock-outs and synthetic lethal mutations, genetic variation, in vitro transcription factor binding, and in vivo protein binding to native chromatin. Because improved integration of oligonucleotide design, data acquisition, analysis, and the ability to share data with other laboratories must assume a high priority in today's post-genomic research setting, we are developing software that can quickly find unique oligonucleotides for hundreds or thousands of genes or DNA segments simultaneously. This software is being integrated into a relational database package utilizing emerging MIAME/MAGE standards for microarray data. Data from other high-throughput techniques, such as DNA sequencing, genotyping, and proteomics, can be ported into this system, and links to analysis software developed at the ISB or at other institutions will be incorporated. The goal of this project is to make a fast, flexible ink-jet array system available to the scientific community and to provide software that enhances the functionalities of the system through quality control, data collection and analysis, and graphic visualization of the complex data-sets generated by the arrayer.

Lee, Zhenghong Case Western Reserve University Quantitative FDG-PET For Hepatomacellular Carcinoma CA095307 4 years NCI

Abstract: This research is part of a larger, long-term effort to aid the development of radiological interventions based on in vivo multimodal imaging, mainly quantitative PET imaging, for cancer diagnosis and treatment. These image-guided interventional procedures include biopsy, minimally invasive drug delivery and tumor ablation. Currently, PET imaging using [18F]-fluorodeoxyglucose (FDG) for tumor-detection does not utilize its full clinical potential. Accordingly, our research project addresses the correlation between PET measured tracer transport rate parameters (for FDG in this project) and histopathologically evaluated cancer prognostic factors. These cancer prognostic factors include pathologic type and grade, proliferation rate, p53 expression, etc. They are more useful for patient categorization, more crucial for treatment decisions, and more important for early assessment of the outcome of interventional procedures because they provide cellular level information as well as the microenvironment of the cancer. Our initial objective is to establish the woodchuck model of Hepatocellular Carcinoma (HCC) for quantitative FDG-PET imaging. Through multimodal imaging, we will align images from PET, CT and histology. Using functional analysis, we will correlate FDG transport parameters with cancer prognostic factors. We have assembled a strong research team with the needed expertise to accomplish our goal this BRG project. We hope that such correlation, if established, will lead to more successful cancer prognosis and early prediction of treatment efficacy and will thus have a huge impact on patient management. The methodology developed in this research can be applied to other tracers and used for other problem-specific research.

Levy, Robert J Children's Hospital Of Philadelphia Gene Delivery Stents HL072108-01 4 years NHLBI

Abstract: Expandable metallic stents have been successfully utilized to relieve coronary arterial obstruction During this past year in the United States more then five hundred thousand coronary stents were used. Furthermore, there has been increasing interest in drug delivery stents to prevent restenosis following stent angioplasty. Our laboratory has pioneered the use of stents as platforms for gene delivery systems for arterial wall gene therapy. In this research program, we will address the following hypothesis: Gene therapy for in-stent restenosis and stabilization of vulnerable plaque can be achieved with a gene delivery stent. Our gene delivery stent utilizes antibody-mediated tethering of replication defective adenoviral gene vectors; this results in enhanced site specific ta'ansgene expression, and a highly localized biodistribution restricted to the site of stent deployment. Aims Aim 1: Polyaminobisphosphonate-steel interactions with subsequent polyamine and antibody binding: Formulation and Characterization. Bisphosphonate chemosorption will be the basis for a molecular surface modification of the steel stents either directly or with amplifying polyamines, to permit the covalent binding of anti-adenoviral antibodies to amino groups using bifunctional crosslinking, thereby enabling vector tethering. Aim 2: Formulation and characterization of the steel-PAABP-antibody gene delivery system: Ceil culture studies. Arterial smooth muscle cell cultures will be used as a model system to investigate the mechanism of gene delivery, fi-galactosidase (LacZ) and Green Fluorescent Protein (GFP) will be used as the reporter genes for these experiments. Aim 3: In vivo efficiency and anti-in-stent restenosis efficacy. Pig coronary stent studies will use the optimal formulations based on Aims 1 and 2. Reporter studies (LacZ) will focus on in vivo efficiency and biodistribution of vector. The therapeutic gene will be Fas-Ligand, a pro-apoptotic protein with established efficacy for restenosis. The chief therapeutic endpoint will be extent of inhibition of neointimal formation.

Lewis, Randolph V   University Of Wyoming   Designing Spider Silk Proteins As Novel Biomaterials   EB000490   3 years   NIBIB

Abstract: Spider silk, which has been evolving for over 450 million years, has a tensile strength greater than steel and elasticity greater than nylon. A number of spider silk genes have been cloned and sequenced revealing specific amino acid motifs that have been conserved for over 125 million years. The key element in taking the next step toward generating biobased materials from spider silks will be to move from the current descriptive data to predictive knowledge. No one has systematically varied the sequence motifs in the spider silk proteins and determined how this influences the mechanical properties of the resulting fibers. These experiments will provide the predictive knowledge enabling the design of materials with very specific elastic and strength properties for each different medical application. This proposal is designed to test three basic hypotheses and engineering concepts. 1) Amino acid sequence motifs from spider silk assembled into a protein can be used to create self-assembling elastic materials. 2) The elasticity of the materials will be proportional to the number of elastic motifs. 3) Varying the sequence of the elastic regions will vary elastic (Young's) modulus. A brief work plan is described here. 1) Genes will be constructed with variations in the number and sequence of elastic motifs, based on naturally occurring spider silk sequences and the encoded proteins expressed in a suitable host system, 2) Each of these different proteins will be used to make both fibers and thin films. 3) The films and fibers will be tested for their mechanical properties. The properties to be measured will be tensile strength, elasticity (recoverable elongation), total elongation, energy to break and elastic modulus, 4) The structure of the protein in solution, films and fibers will be determined by Fourier transform infrared spectroscopy (FTIR) and circular dichroism (CD) and by solid state NMR. 5) The elasticity and elastic modulus data will be correlated with the number and sequence of each type of motif to produce a prediction algorithm for elastic and other materials properties. This project is highly significant for several reasons. First it will provide a basic understanding of elasticity and tensile strength in spider silk proteins, Specifically, it will reveal what controls the amount of elasticity and elastic modulus and if these two factors can be varied in a predictable way. Second this project will advance our ability to use spider silk as a biomaterial. If our hypotheses are correct we will learn how to control the elasticity and other materials properties by controlling the protein sequence. Possible applications of spider silk range from artificial ligaments and tendons to bandages for burns to composite materials for multiple applications.

Li, Alexander D   Washington State University   Nano Accordions For Probing Biomolecular Interactions   GM065306   5 years   NIGMS

Abstract: Protein and deoxyribonucleic acid (DNA) interactions are ubiquitous and play vital roles in controlling functional cellular systems and abnormal cell transformations (e.g., tumor progression). Upon binding, the DNA and/or proteins frequently undergo conformational changes due to the requirement of new complex formation. We hypothesize that these "molecular mechanics" can be probed with a man-made molecular accordion consisting of a string of fluorescent chromophores linked with foldable hinges. Molecular conformation changes will exert forces on the accordion, thereby tuning the fluorescent emission colors. As a first step, we will use solid phase synthesis to construct molecular accordions consisting of fluorescent chromophores and DNA sequences known to promote protein binding. The accordion-protein interactions will be carried out in vitro with wellcontrolled parameters. Our specific aims are to design and synthesize molecular accordions with multiple fluorescent chromophores on DNA and to apply fluorescent spectroscopy and microscopy in controlled conditions to gauge protein-DNA interactions. In particular, we will start with HMGA1 protein, which is a diagnostic marker for neoplastic transformation and metastatic potential of many type cancers. In this project, we plan to determine the parameters that define the scope of the formation of protein-DNA-accordion complex; these parameters will be essential for proposed future in vivo applications of molecular accordions. Our long-term objectives are to use these molecular accordions to probe regulatory events in vivo and to further our understanding of abnormal cell transformations underlying molecular mechanism of diseases.

Madsen, Ernest L University Of Wisconsin Madison Phantoms For Use In US And MR Elasticity Imaging EB000459 3 years NIBIB

Abstract: Elastography has been under development during the last decade and is recognized as having great potential as an emerging major tool for breast and prostate cancer diagnosis. It may also play an important role in other areas such as monitoring tumor ablation therapy and intravascular plaque classification. Initial clinical trials are under way and there is a great need for temporally stable heterogeneous phantoms to enable vigorous development of elastographic hardware and software. The overall objective of this grant is to develop tissue-mimicking (TM) materials and phantoms with long-term stability of elastic, ultrasonic and MR properties. These materials are to be used for developing and performance testing of elastography (elastic imaging) systems. One type of phantom will be used in calibration, standardization and performance assessment of elastography machines and the various algorithms used to create elastograms. Another type of phantom will be used to assess the accuracy of an instrument (NanoIndentor XP(R)) dedicated to in vitro mm-scale mapping of the Young's modulus for thin slices of normal and abnormal tissue specimens. A third type will consist of anthropomorphic phantoms with precisely known geometries and physical properties. These will be made in order to allow a more realistic challenge to elastographic systems under development; such phantoms will include breast and prostate relative to cancer diagnosis and other organs such as the liver regarding tumor ablation. Long-term stability of newly developed materials and heterogeneous phantoms will be completed by monitoring elastic, ultrasonic and MR properties and internal structural configurations of heterogeneous phantoms. Refined heterogeneous performance phantoms and anthropomorphic phantoms will be developed iteratively with assessments and advice from collaborators who are active researchers in ultrasound and MR elastography.

Messersmith, Phillip B Northwestern University Bioinspired Synthesis Of In-Situ Gelling Biomaterials DE013030 5 years NIDCR

Abstract: Transglutaminase enzymes are ubiquitous Ca2+-dependent enzymes that catalyze the formation of crosslinks between glutamine and lysine residues of proteins. Extensive transglutaminase-mediated crosslinking of soluble proteins is believed to be responsible for rapid physical gelation of certain biological fluids. A common biological strategy for regulating the activity of transglutaminase enzymes is control of intracellular and extracellular Ca 2+ concentration, mediated by lipid bilayer membranes. Stimuli-responsive synthetic lipid vesicles offer a unique opportunity to regulate transglutaminase-mediated gelation by sequestering and then releasing enzyme-activating ions such as Ca 2+. We hypothesize that Ca 2+ release from temperature or light sensitive liposomes can be used to trigger TG-mediated crosslinking of peptide-modified polymers to form hydrogels suitable for use as tissue adhesives and for injectable tissue engineering. In this study, combinatorial chemistry will be employed to synthesize large peptide libraries from which short peptide substrates of transglutaminase enzymes will be identified. The peptide substrates will be covalently linked to biocompatible polymers, and the TG-catalyzed crosslinking of the polymers into hydrogels will be studied in an effort to formulate injectable solutions that undergo rapid gelation in situ. Stimuli-responsive liposomes will be utilized to trigger calcium activation of enzyme-catalyzed gelation with the goal of developing thermal and light triggered gelation for clinical use. The tissue adhesive potential of these hydrogels will be assessed by measuring the force required to separate articular cartilage surfaces bonded together by in-situ formed hydrogels, and in vitro and in vivo studies will be performed to evaluate the potential of chondrocyte-containing injectable polymer hydrogels to support the formation of cartilage tissue.

Mizaikoff, Boris   Georgia Institute Of Technology   Scanning Probes To Image Cellular Signaling Processes   EB000508   5 years   NIBIB

Abstract: The present project proposes an innovative, multidisciplinary approach to the investigation of cell communication processes at the molecular level. In this project, novel, innovative and interdisciplinary research is emphasized with a focus on the application of microfabricated integrated scanning nanoprobe and nanobiosensing systems. Scanning probe microscopy (SPM) techniques provide powerful means for obtaining chemical, topographical and optical information with high spatial resolution. Each technique - atomic force microscopy (AFM), scanning nearfield optical microscopy (SNOM) and scanning electrochemical microscopy (SECM) - is designed to provide specific information based on interaction with the sample surface in the nearfield regime. SECM is especially interesting for biochemical/biological investigation, since this technique provides information on electrochemical, chemical and biochemical (re)activity at sample surfaces. The proposed multifunctional tool based on combination of scanning probe techniques will provide simultaneous information with a high selectivity for individual transmitters, high temporal resolution of changes in transmitter concentration, and high spatial resolution to distinguish which cells are releasing transmitter molecules, information required for in-situ investigations of complex biological systems and heterogeneous matrices. Many pathological events involve disruption of chemical communication between cells, most notably in the central nervous system, lungs, and kidneys. An important component of cell communication is the exocytotic release of transmitters from a variety of cells (e.g., the presynaptic terminal), their diffusion across between cells (e.g., a cross the synaptic cleft to the postsynaptic membrane) and the selective binding to suitable receptors, which lead to the initiation of cellular signalling events specific to the transmitter and the receptor. Little is known about the formation of secretory granules and the characteristics of transmitter release, which is frequently altered during physiological or pathophysiological conditions. Hence, detailed knowledge and multiparametric analytical assessment of exocytotic events will help understand the underlying mechanisms of cellular communication. Multifunctional microfabricated SECM-AFM, SECM-SNOM and SECM-AFM-SNOM with integrated nanobiosensors will provide simultaneous topographical, optical/fluorescence and electrochemical information at nanometer resolution for the first time. We will apply these combined multifunctional techniques in combination with tip-integrated nanobiosensors to a relatively simple model biological system to demonstrate the feasibility of the approach for measuring biologically relevant transmitter molecules. The model system will involve the exocytotic release of ATP from a monolayer of epithelial cells in culture. This system is a good model for a variety of epithelial tissues in mammals and the question of the amount and regulation of ATP release is a contemporary issue following the hypothesis that abnormalities in ATP release could play a significant role in the pathophysiology of cystic fibrosis (CF). Quantitative theoretical models and simulations of multifunctional scanning probes in order to facilitate interpretation of electrochemical results and imaging data will complement the proposed tasks.

Muthukumar, Murugappan   University Of Massachusetts Amherst   Modeling Macromolecular Transport Through Channels   HG002776   3 years   NHGRI

Abstract: We propose to develop the macromolecular modeling needed for a fundamental molecular understanding of how electrically charged polymer molecules move through protein channels. Stimulated by prospects of direct high-speed detection of sequences in single polynucleotide molecules, and of stochastic sensing of single macromolecular analytes, very exciting single-molecule electrophysiology experiments have recently been reported. The results of these experiments are very puzzling and require an understanding of polymer physics, in combination with descriptions of chemical specificities. We propose to implement polymer physics concepts valid at large length and time scales, in conjunction with Brownian Dynamics simulations accounting for details at smaller length and time scales. The present proposal addresses a fundamental understanding of (1) how DNA/RNA molecules move through alpha-hemolysin channels under an electric field and computation of ionic current of channels with macromolecular transport, (2) origin of discrete conductance states and conformations of polymer tethers engineered into protein pores, and (3) development of coarse-grained models for RNA-carrying transport factors and their transport through nuclear pore complexes. Our unique combination of theory, simulations, and collaborations with active experimentalists, will have a direct and profound impact on understanding of signal transduction, high-speed sequencing of DNA/RNA and proteins, screening of biological warfare agents, pharmaceutical diagnostics, and macromolecular aspects of diseases and their control.

Okamura, Allison M   Johns Hopkins University   Haptic Feedback For Robot-Assisted Surgical Systems   EB002004   4 years   NIBIB

Abstract: Cardiac surgery is traditionally performed through a median sternotomy, providing optimal access to all cardiac structures and great vessels, but generating significant disfigurement and pain for the patient. The advent of robot-assisted minimally invasive surgery (MIS) holds great promise for improving the accuracy and dexterity of a surgeon while minimizing trauma to the patient. However, clinical success with robot-assisted cardiac MIS has been marginal; improved patlent outcomes and mitigated costs have not been proven. We hypothesize that this is due in large part to the lack of haptic feedback presented to the surgeon. Without the sense of touch naturally used in surgical tasks such as fine suture manipulation, surgeon performance is jeopardized. The general objective of the proposed research is to acquire and use haptic information during robot-assisted minimally invasive surgery, with a focus on manipulation of fine sutures. It is anticipated that this approach will offer two main benefits over current systems: (1) forces will be fed back to the user in real time, directly or through sensory substitution, improving task performance, and (2) haptic information will be used to create automatic virtual fixtures that assist the surgeon, e.g., by prevention of excessive applied suture forces and maintenance of constant retraction forces. The specific aims are: (1) to understand the sensing requirements for minimally invasive surgical tasks, (2) to test different modes of haptic feedback in robot-assisted minimally invasive surgical environment, (3) to create virtual fixtures that augment and improve the execution of surgical tasks, and (4) to apply feedback of haptic information during phantom experiments, creating a direct path to clinical applications. These specific aims contribute to a Iong-term research plan for using haptic information in robot-assisted minimally invasive surgery. The proposed work represents the development of operative technology that can provide significant improvements in patient outcomes, as well as Iay the groundwork necessary to address several other exciting research issues such as beating-heart (off-pump) procedures, procedure and tissue models, and virtual environments for training.

Ostermeier, Marc A   Johns Hopkins University   The Combinatorial Design Of Protein Molecular Switches   GM066972   5 years   NIGMS

Abstract: Protein molecular switches functionally couple external signals (such as ligand binding) to functionality. Molecular switches have a wide variety of potential health related applications including the regulation of gene transcription, the modulation of cell signaling pathways, targeted drug delivery, drug transport, the creation of conditionally active toxic proteins, and the creation of molecular biosensors. Despite their great potential, the creation of protein switches has not been extensively explored, in part due to the paucity of general strategies for their engineering. Using combinatorial methods that integrate biological, chemical and engineering approaches, molecular switches will be engineered by a novel strategy called 'combinatorial domain insertion' using model proteins in 'proof-of-principle' experiments. In combinatorial domain insertion, two genes are fused such that one is randomly inserted within the other. In the model system chosen, Gene A codes for a binding protein that undergoes a conformational change in the presence of a signal (e.g. ligand binding). Gene B codes for a protein to be controlled (e.g. an enzyme). From these libraries, fusion proteins will be identified that functionally couple the two domains' functions (e.g. ligand binding modulates the enzyme's activity). The functional coupling is hypothesized to result from ligand-dependent conformational/stability changes in protein A that affect the activity of protein B. Representative switches obtained will be kinetically and structurally characterized. Through the systematic analysis afforded by a combinatorial approach and the biochemical and structural characterization of the switches created, models of the mechanism of switching will be developed and tested experimentally with the goal of elucidating general) principles that can be applied to the creation of molecular switches for biomedical applications.

Patzer, John F University Of Pittsburgh At Pittsburgh Bound Solute Dialysis DK063244 4 years NIDDK

Abstract: The American Liver Foundation estimates that one in ten people has some form of liver disease and that 26,000 people die each year from liver disease. In the year 2000, 18,137 people were listed for liver transplantation, only 4,934 cadaveric donor liver transplants were made, and 1,636 patients died while awaiting transplant. A medical need for a treatment modality that can "bridge" patients to transplant or slow or reverse the progression of liver disease clearly exists. Even better would be a new standard of care treatment that can be easily employed by any hospital at early stages (Parson's encephalopathy grade 1 and 2) of liver failure that would retard or prevent progression to acute liver failure that requires OLTx. Bound solute dialysis (BSD), practiced heuristically by the MARS and Biologic-DT approaches, offers the potential of a new standard of care treatment modality. Improvements in such heuristic practice require a theoretical approach that encompasses the underlying thermodynamics and transport phenomena. Such an analysis indicates that BSD is both more robust and more easily employed than expected from the heuristic approaches practiced here-to-fore. Experimental observations follow the theoretical predictions. The objective of this research proposal is to expend upon the preliminary studies in order to define what is necessary to bring BSD to the status of an easily employed standard of care treatment for liver disease that can be practiced with minimal requirements by any hospital or clinic. This will be accomplished by expanding the theoretical approach to encompass other forms of BSD, experimental validation of modeling predictions, development of an animal model of chronic liver failure, and preclinical evaluation of the various modes of BSD with the animal model.

Pauly, John M Stanford University Comprehensive Assessment Of Valvular Function With MRI HL074332 4 years NHLBI

Abstract: The goal of this proposal is to develop and validate a comprehensive examination of valvular heart diseases. Valvular heart disease affects approximately 10% of the general population in the United States. Over the past 20 years, valvular diagnosis has undergone a revolution due to advances in cardiac ultrasound. However, ultrasound has inherent limitations with respect to tissue characterization, spatial resolution, and the need for acoustic windows. Particularly difficult are the evaluation of valvular morphology, quantitation of valvular stenosis and identification and quantitation of valvular regurgitation. The examination of valvular heart disease includes the assessment of valvular morphology, cardiac output, intracardiac pressures, ventricular volume and volume regurgitations. Magnetic resonance imaging (MRI) is potentially the most appropriate technique for addressing all of these areas in a single examination. Current MR techniques for valvular imaging suffer from poor temporal and spatial resolutions, require prolonged acquisitions, and frequently require laborious post processing. As a result, there is a gap between what is scientifically feasible and what is currently applied clinically. Our goal in this proposal is to eliminate this gap between the potential of MRI and current clinical practice. Our group has pioneered many of the components that will be useful for the diagnosis of valvular heart disease, including real-time imaging, real-time color flow, and MR Doppler. In this proposal we will integrate and extend these components along with new developments to provide an integrated and comprehensive assessment of valvular function.

Pertsov, Arkady M   Upstate Medical University   Depth-Resolved Imaging Of Myocardial Excitation   HL071762   4 years   NHLBI

Abstract: Understanding the mechanisms that underlie abnormalities of electrical conduction in the heart is the key to the development of effective antiarrhythmic therapies. During the last decade, significant progress has been made in imaging electrical excitation waves in the heart using voltage-sensitive fluorescent dyes. However, until recently imaging that uses voltage-sensitive dyes was limited primarily to the epicardial surface. Therefore the design of tools for visualization of electrical activity inside the myocardial wall is likely to have a major impact on the field. The ultimate goal of the proposed studies is to develop a new optical imaging technology for detecting sources of arrhythmia hidden inside the myocardial wall. The new technology will combine methods of diffusive optical tomography with specific knowledge of electrical processes in the heart and their characteristics. The first major step towards our main objective will be to create a family of realistic tissue-specific and dye-specific computer models for reconstructing optical images from 3D distributions of the transmembrane potential in myocardial tissue (forward problem). Using this methodology, termed below virtual optical imaging (VOI), we will create a web-accessible library containing the optical signatures of major types of activation patterns. The solution of the forward problem will provide key elements for solving the inverse problem - reconstructing 3D distributions of the transmembrane potential from optical signals. The specific aims of the project are: 1) To develop a realistic model of light transport in myocardial tissue based on direct measurements of light absorption and scattering. 2) To couple the light transport model to a model of electrical wave propagation. The combination will predict voltage-dependent optical signals during normal propagation and 3-dimensional reentrant activity. 3) To develop and validate experimental techniques and deconvolution algorithms for imaging intramural sources of excitation including ectopic foci producing radial spread of excitation, and scroll wave filaments - the organizing centers of 3D reentrant activity in myocardial tissue. Successful completion of the project should significantly improve ones ability to interpret optical recordings and will ultimately enable depth-resolving detectors for imaging the intramural sources underlying the most lethal arrhythmias.

Pikov, Victor   Huntington Medical Research Institutes   Virtual Stroke Model For Study Of Bladder Hyperreflexia   EB000518   3 years   NIBIB

Abstract: Stroke is the third leading cause of death in the United States and produces more than $30 billion yearly in health care costs. About 30 percent of stroke victims are permanently disabled with a variety of impairments. Voiding dysfunction is important factor in long-term morbidity of stroke patients and is manifested as frequency, urgency and urge incontinence. These symptoms are generally a result of bladder detrusor hyperreflexia. The presence of bladder detrusor hyperreflexia is correlated with infarctions in the frontal cortex (frontoparietal lobes). Similarly, experimental frontal cortex infarction in animals produces bladder hyperreflexia within a short time after infarction onset. It has been demonstrated that stroke may produce corticospinal disinhibition, which then leads to bladder hyperreflexia. This study aims to develop an animal model of "virtual stroke" in order to reliably and repetitively produce bladder hyperreflexia. Studies in human volunteers indicate that low-frequency magnetic stimulation of the cortex produces stroke-like symptoms and, in particular, considerable corticospinal disinhibition. Development of the animal model will allow us to study spinal cord mechanisms of "virtual stroke"-evoked bladder hyperreflexia and to attempt its suppression by repetitive stimulation of bladder efferents. We hope that this project can lead to development of a better treatment of stroke-related bladder dysfunction.

Pine, Jerome   California Institute Of Technology   The Neurochip: A New Tool For Studying Cultured Networks   NS044134   4 years   NINDS

Abstract: The primary goal of this project is to develop a prototype of a silicon-based structure, the "neurochip" which will support the growth and development of cultured neural networks and will greatly enhance the ability of researchers to study them. The structure will be an array of 61 "wells" into which dissociated neurons can be placed, and which will hold each of them in proximity to an extracellular electrode. The wells will allow process outgrowth of axons and dendrites and be closely spaced so as to support synaptic connectivity of the neurons to form a network. The prototype can be scaled up to larger networks, and the fabrication method will be compatible with on-chip CMOS electronics for on-chip processing, control, and wireless communication. The electrodes will provide the capability to stimulate and record from any chosen neurons of the network non-destructively, supporting studies of the network connectivity over time; of patterns of spontaneous activity;, and of activity-dependent effects resulting from stimulation. A second goal of the project is to utilize neurochips to perform an initial series of experiments which will reveal the development of connections, the effects of stimulation on remodeling the network and the creation of "learned" responses. These will provide unique knowledge of network behavior, in detail, beyond what is now possible with available techniques in vivo or in vitro. In addition, neurochip networks have the potential to reveal pharmacological effects relevant to drug design and development, and also to exhibit the effects on nervous system function of genetic defects.

Pipe, James G   St. Joseph's Hospital And Medical Center   Advances In Phase-Contrast MR Angiography   HL067821   3 years   NHLBI

Abstract: The broad, long-term objective of this project is the advancement of MRI as a tool for vascular diagnosis. The specific goal is to improve a method of MRI known as phase-contrast MR angiography (PC-MRA) with several novel contributions that make it more practical to use for both clinical diagnosis and basic science research, and that improve the accuracy and breadth of information obtained from this method. PC-MRA has a unique potential among all imaging modalities to yield reproducible, quantitative four-dimensional (space + time in the cardiac cycle) information about blood flow velocities and patterns. This information has potential in assessing vascular morphology in a clinical setting, understanding the hemodynamics that may contribute to pathogenic processes, predicting the risk of atherosclerosis and of acute events such as stroke and aneurysm rupture, and aiding in the design of vascular grafts and methods of graft placement. Despite its potential, PC-MRA is not routinely used currently, in part because of long imaging times and its inability to accurately characterize disordered flow. Work proposed in this application will attempt to advance PC-MRA in four key areas of development: 1. A simple, robust method of estimating wall shear stress and flow disorder from PC-MRA data will aid in quantification of wall shear stress, and aid in visualization of areas with flow disorder. 2. A rapid method for data acquisition will be developed and implemented. This, along with new methods for data reconstruction, will add flexibility to data collection and reduce artifact. 3. Expansion of a previously proposed technique will increase greatly increase data SNR, subsequently improving vessel conspicuity as well as the precision of flow velocity measurements. 4. Data processing will improve the accuracy and information content of the reconstructed images.

Puleo, David A   University Of Kentucky   Biomaterials With Rationally Immobilized Growth Factors   AR048700   4 years   NIAMS

Abstract: Ideally, implants should be designed with the ability to promote specific biological responses. In the case of bone-contacting materials, this would mean that, immediately following implantation, the devices would induce cells of the osteoblastic lineage to form bone on or in close proximity to the biomaterial. Stable fixation of the implant and better integration into the tissue would be the result. The overall goal of this research is to develop biomolecular surface modification strategies that induce specific cell and tissue responses. For example, stimulation of tissue regeneration, in which the implant becomes integrated into the tissue, is more desirable than repair, in which the implant is generally walled off with a fibrous capsule. It is postulated that bone-contacting materials possessing surfaces with osteotropic biomolecules in a specific presentation can control initial cellular events at the tissue-biomaterial interface. In Specific Aim 1, bis-hydrazide-derivatized poly(D,L-lactide-co-glycolide) coatings, microspheres, and porous scaffolds will be developed for use in site-directed immobilization of osteotropic growth factors. Surfaces with different lengths and surface densities of bis-hydrazide molecules will be produced. In Specific Aim 2, rational schemes will be designed and implemented for site-directed immobilization of growth factors on the materials developed in Aim 1. Recombinant DNA techniques will be used to introduce modifications to the amino termini of insulin-like growth factor I and bone morphogenetic protein 2. These unique sites will allow the growth factors to be immobilized in an orientation that maximizes interaction with cell surface receptors. In Specific Aim 3, in vitro and in vivo biological activity of the rationally modified biomaterials will be determined. Cell cultures will also be used to assess activation of the appropriate cell surface receptors by the immobilized growth factors. It is hypothesized that site-directed immobilization of growth factors will yield higher specific bioactivity and, therefore, will result in greater cell and tissue responses (specifically bone formation) compared to randomly immobilized protein. The findings of these studies will impact the development of bone-contacting biomaterials having the ability to induce desired interracial responses.

Qin, Yi-Xian   State University New York Stony Brook   Bone Fluid Flow And Its Regulatory Role In Adaptation   AR049286   4 years   NIAMS

Abstract: Musculoskeletal complications, such as osteoporosis and aging related osteopenia, are major societal and health problems. Load-induced intracortical bone fluid flow is proposed as a critical mediator in initiating and regulating bone surface and osteonal adaptation. Using oscillatory pressurized marrow fluid flow stimuli, the physiological fluid stimulus was found to initiate new bone formation and reduce intracortical bone porosities caused by disuse, even in the absence of direct tissue strain. The new bone formation and inhibition of resorption were found to correlate with quantified flow parameters, i.e., fluid pressure gradients. This flow initiated bone adaptation occurs at a specific frequency range, i.e., 20-30 Hz, and is interdependent with the dose of anabolic fluid pressure. While bone remodeling was demonstrated to be sensitive to high strain frequency and low intensity physiological loading, the role of fluid flow perhaps explains, at least in part, the cellular response mechanism to anabolic stimuli. In the work proposed, we will examine the general hypothesis that bone fluid flow, mediated at specific physiological magnitudes and high frequencies, promotes osteogenic adaptation. Indeed, improving our understanding in which mechanical signals influence the temporal and spatial dynamics of bone remodeling may help to devise a biomechanically based intervention for treating osteoporosis, accelerating fracture healing or promoting bony ingrowth into prostheses. In this revised application (1-R01-AR049286-01), the goal will be achieved by a series of sub-hypotheses and specific aims: (1) The role of anabolic fluid flow, driven by daily intramedullary pressure (IMP), can initiate surface adaptive response and inhibit intracortical bone loss in a disuse bone. The remodeling response will be evaluated in a disuse in-vivo model in the absence of matrix strain following 4-week exposure of a short period of daily stimuli, consisting of a series of frequencies (0.5,1,5,10,20 & 40 Hz). (2) Osteogenic response to anabolic fluid flow stimuli is fluid pressure sensitive associated with the rate/frequency of loading. The anabolic potential response to hydraulic intensity will be evaluated in a disuse model following 4-week of daily ImP at 10, 20 and 80 mmHg with 1,5, and 20 Hz. (3) The potentials of fluid flow initiated adaptation are interdependent with specific fluid components, i.e., pressure gradient and fluid shear stress, which are responsible for restoring or inhibiting bone loss and new surface bone formation. A poroelastic finite element analysis will be developed, which will evaluate the correlation between fluid flow and resultant adaptation. (4) The osteogenic potentials response to fluid flow stimuli is initiated by osteoblastic activation of bone lining cells, following a daily but short duration (e.g.,

Rabin, Yoed Carnegie-Mellon University Thermo Mechanical Stress In Cryopreserved Blood Vessels HL069944 4 years NHLBI

Abstract: Cryopreservation technologies represent a potential long term and minimally damaging method to preserve both native and engineered tissues. Conventional cryopreservation of allogeneic veins involving freezing is currently being used clinically, but in vivo studies using these grafts in both animal models and patients have demonstrated poor long-term patency rates. An alternative approach to cryopreservation involving vitrification that avoids the hazards of ice formation leads to a markedly improved vascular product in terms of both structure and function. Vitrification (vitreous means glassy in Latin) is essentially the solidification of a supercooled liquid by adjusting the chemical composition and cooling rate such that the crystal phase is avoided. This new preservation technology is now being scaled up for application to clinical specimens and ultimately engineered blood vessels. Nevertheless, additional hazards related to thermo-mechanical stresses in bulk vitrified specimens must be avoided for successful cryopreservation of tissues. Our long term goal is to reduce the destructive mechanical stresses induced during cryopreservation of tissues in general, and of blood vessels in particular. The purpose of this research is to develop engineering tools and to characterize the level of thermo-mechanical stresses in bulky cryopreserved tissues and thereby devise techniques to reduce, or circumvent, these stresses and develop improved methods of long term storage of both native and engineered vascular grafts. The specific aims are to undertake a systematic study of thermo-mechanical stresses in cryopreserved blood vessels by measuring thermal expansion and stress-strain relationships. The measured parameters with appropriate mathematical modeling and computers simulations, will provide guidelines for minimizing the thermo-mechanical stresses and reduce the potential of fracture formation during cryopreservation. Although the experimental work in this study is targeted to blood vessels, the results of this study could be expanded and become useful for a wide variety of cryopreserved natural tissues and engineered constructs.

Raftery, M Daniel Purdue University West Lafayette Multi-Coil, Multi-Sample Magnetic Resonance RR018294 4 years NCRR

Abstract: Despite its importance as a versatile tool in many chemical analyses, nuclear magnetic resonance (NMR) spectroscopy suffers from its poor sensitivity and sample throughput capabilities. This proposal describes a program to make a significant improvement in the throughput of NMR measurements. A new approach, Multiplex NMR, can simultaneously measure the NMR signals from multiple samples. The proposal focuses on five areas of fundamental research to develop this new technology to the point where it can be useful for a wide range of NMR measurements of interest to the biomedical community. Specific Aims: 1. Develop and test methods for high speed data acquisition on combinatorial peptide libraries 2. Develop 2D NMR methods for simultaneous analysis of multiple samples and faster LC/NMR 3. Increase sensitivity and parallelism to 8 or more samples using micro-fabrication methods 4. Improve sensitivity using inline sample pre-concentration methods 5. Explore the use of parallel NMR coils for solvent suppression and NMR difference spectroscopy. The long-term goal of this research is to provide order of magnitude improvements in the throughput of single- and multi-dimensional NMR measurements. Applications in areas such as combinatorial chemistry, high throughput screening, organic synthesis and biological chemistry will benefit from the development of the approach described in this proposal. Two specific biomedical applications of the new technology involving the analysis of combinatorial peptide libraries and the study of protein-ligand interactions are described.

Raylman, Raymond R   West Virginia University   A Positron Emission Mammography/Tomography Biopsy Device   CA094196   4 years   NCI

Abstract: Detection of cancer in the dense breast is a significant challenge for x-ray mammography. Similarities between the density of lesions and surrounding breast tissue, in addition to x-ray opacity, limit effective detection of small to medium sized tumors in these patients. To address this concern, nuclear medicine-based imaging techniques, such as positron emission tomography (PET) used with 18F-Fluorodeoxyglucose (FDG), have been applied with some success to breast imaging. These methods rely upon differences in metabolic activity between tumor and normal tissue, instead of density contrasts for creating images. Although these techniques have demonstrated some potential in detecting breast lesions in dense breasts, their limited spatial resolution and specificity does not warrant their sole use for making diagnoses. The most effective method for diagnosis remains histological evaluation of tissue samples acquired from biopsy. In many cases stereotactic core biopsy of suspicious lesions guided by x-ray mammography is an effective and minimally-invasive method for performing these procedures. In the case of lesions optimally detected in a dense breast with PET, however, x-ray techniques may not be optimal for biopsy guidance. In response to this need, we propose the construction of a device that will be capable of acquiring high resolution images of the breast using PET techniques. These images will then be used to assess radiotracer uptake and guide the placement of a biopsy needle into suspicious lesions. Image-based verification of needle positioning will be accomplished by acquiring a set of stereotactic planar, positron emission mammography (PEM) images. This dual modality system called, PEM-PET, will be capable of performing three main tasks: lesion detection, radiotracer quantitation and lesion localization. The apparatus will consist of two sets of rotating, large-area planar pixelated scintillator arrays. The use of rotating planar detectors was adopted (instead of the more intuitive ring geometry) to facilitate access to the breast during biopsy procedures, while permitting acquisition of PEM and PET images. The choices of scintillator material (Lutetium Oxyorthosilicate) and detector pixel size were based on computer simulation studies and model observer studies. Development of the system will include the construction of the detector units, and the creation of tomopraphic and planar image reconstruction methods. The PEM-PET system will be optimized and evaluated with task-dependent metrics obtained from model and human observer studies, and a pre-clincal trial. At the completion of this project an apparatus for the effective detection and diagnosis of lesions for use in the subgroup of women with difficult to image breasts will have been developed and evaluated.

Rothman, Steven M   Washington University   New Strategies For Neocortical Epilepsy   NS042936   5 years   NINDS

Abstract: The treatment of many human epileptic syndromes remains unsatisfactory. While anticonvulsant medications allow about 75% of epileptics to achieve excellent seizure control, the remaining 25% of patients suffer from a combination of continued seizures and medication toxicity. It is unlikely that a single medical breakthrough will provide a cure for all of these refractory patients. Focal neocortical epilepsies have proven particularly difficult to manage. While some respond to anticonvulsants, a large fraction remains intractable to medical therapy. This group can respond to cortical resection, but surgical management is problematic. Exact identification of the epileptogenic focus can be complicated and there is a risk of unanticipated, irreversible neurological deficits after resection. Focal cortical cooling has the potential to improve the evaluation and treatment of this epileptic subgroup. The aims of the experiments described in this application are to investigate the potential of focal cooling with thermoelectric (Peltier) chips to rapidly terminate chronic seizure discharges, determine the degree of cooling required to stop these seizures, determine whether cooling can prevent seizures, and develop computer programs that recognize and anticipate seizures in "real time". In addition, the potential pathological consequences of cortical cooling will be determined. These experiments represent a necessary first step toward utilizing these techniques for the therapy of human epilepsy. These experiments will utilize models of acute and chronic rodent neocortical seizures and small Peltier devices developed for the microelectronics industry. If Peltier devices can control focal seizures in our models, they will be refined for future experiments to investigate their potential role in mapping and controlling epileptogenic neocortex in man.

Rutkove, Seward B   Beth Israel Deaconess Medical Center   Localized Bioimpedance In Neuromuscular Disease   NS042037   4 years   NINDS

Abstract: This proposal introduces a novel, non-invasive and painless method for the evaluation of skeletal muscle, using a recently developed technique called electrical impedance myography (EIM). The ultimate goal of this research is to refine ElM as a clinical test that can assist in the diagnosis and treatment of neuromuscular disease and in evaluation of muscle change due to disuse. In addition to complementing and potentially replacing the painful test of needle electromyography, ElM will have unique application in the evaluation of muscle weakness in clinical trials research, rehabilitation, and space flight. Our first aim is to establish normal values and confirm the reproducibility of this technique by performing studies on healthy subjects of varying age and fluid status. Second, patients with different disease and disuse states will be evaluated to determine ElM patterns associated with myopathy vs. neurogenic disease and compare the specificity and sensitivity of these patterns to well-established diagnostic methods. Finally, ElM's use as a tool in evaluating neuromuscular disease progression/remission will be assessed and refined. As part of this aim, a prototype device for easy use will be developed and tested in the clinical setting.

Rzigalinski, Beverly A   University Of Central Florida   Nanoparticles As Promoters Of Cell Longevity   AG022617   4 years   NIA

Abstract: The field of engineering has made substantial advances in nanotechnology, particularly in materials science and the molecular construction of nanoscale devices. In this proposal, the PI (Rzigalinski) and co-PI (Seal) have merged the studies of cell biology and nanoscale materials science to intervene in a common biomedical pathology, that being free radical cell damage and aging. We have engineered cerium oxide nanoparticles, 2-20 nm, by a novel non-agglomeration modified sol-gel process and assessed the activity of these particles in a tissue culture model using rat brain cells. Our preliminary data suggests that cerium oxide nanoparticles prolong brain cell longevity in culture, by 2-3 fold. Aged neurons in nanoparticle treated cultures maintained functional synaptic connections and had normal intracellular calcium signaling. Further, cerium oxide nanoparticles reduced hydrogen peroxide (H_2O_2) and UV light - induced cell injury by over 65%. We hypothesize that the unique structure of cerium oxide nanoparticles, with respect to valence structure and oxygen defects, promotes cell longevity and decreases toxic insults by scavenging free radicals. In this proposal we will synthesize and further characterize the physical and chemical properties of cerium oxide nanoparticles, and determine their behavior in physiologically relevant fluids and in the intracellular environment. We will further dissect the role of nanoparticles in cell longevity and determine their mechanism of action. Using microarray technology, alterations in gene transcription in control and nanoparticle treated cells will be during their lifespan. Lastly we will examine the ability of cerium oxide nanoparticles to increase longevity in the fruit fly. These studies will provide substantial insight into the pathology of aging and age-associated disorders and initiate a nanotechnological approach to pharmacotherapy.

Sanders, Joan E   University Of Washington   Assessment Of Tissue Response To Stress   EB000998   4 years   NIBIB

Abstract: The broad long-term objective of this project is a clinically useful tool for assessing, monitoring, and ultimately predicting skin breakdown from mechanical pressure and shear stress as encountered at the residual limb/prosthetic socket interface during ambulation with a prosthetic limb. The Specific Aims are to establish relationships between thermal recovery time (TRT) and tissue bioresponse after a controlled mechanical stress is applied, and then to evaluate TRT as a screening test to predict future skin breakdown in amputee subjects. An additional aim is to better understand how biomechanical features of the applied load that can be controlled by a prosthetist through socket design and fitting decision affect TRT and thus tissue response. To accomplish the aims, first an infrared thermal imaging system is integrated with an existing mechanical loading apparatus to assess TRT after load application. Then well-controlled cyclic pressures and shear stresses are applied to an animal skin model, and quantitative relationships between TRT and physiologic response and between TRT and rapid skin breakdown investigated. The TRT assessment system is then used to monitor amputee subjects in the clinic at monthly intervals to determine if TRT is an effective screening test to predict future skin breakdown. High absolute value TRTs as well as high changes in TRT from one session to the next are expected strong indicators of imminent skin trauma. Then TRT sensitivity to slip vs. stick between the skin and load applicator, a feature that can be controlled by a prosthetist clinically through socket design, is assessed. The health relatedness of this application is knowledge of importance to the development of an instrument to identify imminent skin breakdown in prosthesis users. Such an instrument could have important clinical impact since collected data could be used to modify prostheses or design treatments to avoid imminent skin breakdown. If effective, potentially this technology could be applied to other areas of rehabilitation, for example, shoe design for patients with insensate feet, cushion design for wheelchair users, and mattress design for bedridden patients.

Sanders, Joan E   University Of Washington   Skin Adaptation To Mechanical Stress   AR049644   4 years   NIAMS

Abstract: The long-term objective of this research is to develop therapies to encourage skin strengthening in individuals at risk of skin breakdown from prolonged mechanical stress (e.g. prosthesis users). The specific aims are to develop, evaluate, and use an in vitro skin model system to investigate collagen structural and bioprocess changes that occur in skin adapting to repetitive mechanical stresses, and to apply that insight to an in vivo model to test a new therapy to encourage skin strengthening. For the in vitro model, a surgically-excised pig skin sample is put in culture at an air-media interface within a custom-designed flow chamber. Clinically relevant stresses, those experienced at the residual limb-prosthetic socket interface by lower-limb amputees, are applied to the explant surface for at least a 2-week period using a custom-designed, closed-loop, force-controlled load applicator. Mechanisms of collagen adaptation are investigated to pinpoint specific biomolecules responsible for promoting the adaptive processes resulting in a mechanically stronger structure. A key question to answer is if collagen fibril diameters are increased by adding to existing fibrils or by degrading old collagen and forming new fibrils. It is hypothesized that after a metalloproteinase concentration peak to degrade small existing collagen fibrils, proteoglycans decorin, biglycan, fibromodulin, lumican, and thrombospondin-2 as well as collagen-related integrin expression are upregulated; chondroitin sulfate and collagen V are downregulated; collagen I and III production and cross-linking increase; and new larger collagen fibrils are formed. As a potential therapy to facilitate skin adaptation, a graded-increase stress-application treatment is tested using an in vivo skin model. If started before the metalloproteinase peak has subsided, the treatment is expected to create an architecturally inferior and weak tissue. However, if started after the metalloproteinase peak during remodeling, the treatment should enhance collagen fibril diameter and skin strength. Similar should be the case if the treatment is initiated during the stabilization phase of adaptation, though fibril enlargement and strength should be even further enhanced since the treatment will likely re-initiate the entire adaptation process. If shown to enhance architecture and strength, the treatment regime would have direct clinical applicability. The health relatedness of this proposal is new knowledge that has potential application to the development of novel treatments for persons at risk of skin breakdown. By understanding the process of skin adaptation (natural skin strengthening) at both a cellular and molecular level, therapies to encourage skin adaptation before breakdown occurs can be intelligently pursued.

Scott, Charles P   Thomas Jefferson University   Intracellular Cyclic Peptide Libraries   AI053800   5 years   NIAID

Abstract: The research program described in this application is focused on development of intracellular libraries of cyclic peptides as sources of molecular diversity for drug discovery and chemical genetics. Intracellular peptide and protein cyclization is made possible through circular permutation of inteins (internal proteins). Thus far we have used the technology to elaborate vast, chemically diverse libraries of low molecular weight cyclic peptide in bacterial host cells. To transform these libraries into a comprehensive, cross-cutting technology that can be translated into a variety of biomedical applications, we will: 1) demonstrate that intracellular cyclic peptide libraries can be used to generate high affinity ligands to a selected target receptor; 2) validate peptide cyclization technology in eukaryotic and mammalian cell hosts, which are most relevant for biomedical research; and 3) develop general strategies that enable facile identification of physiological targets that interact with cyclic peptides effectors in cells. My laboratory will pursue a protein engineering approach to these objectives, and implement the improvements as part of a program to discover small molecules that inhibit replication of hepatitis C virus (HCV). We will take advantage of existing libraries in bacterial hosts by creating a high throughput screen to identify potent inhibitors of a heterologously expressed HCV gene product (aim 1). We will optimize intein-mediated cyclization in mammalian host cells and evaluate compatibility of cyclization with signal sequence mediated subcellular localization (aim 2). We will modify the selection marker of the HCV replicon for greater versatility in chemical genetics studies of viral pathogenesis and transfer cyclic peptide inhibitors identified by screening bacterial libraries into the modified HCV replicon to evaluate their effects on viral replication in hepatocytes (aim 3). Finally, We will engineer cyclization constructs for compatibility with two hybrid systems, explore two hybrid systems, expression libraries and affinity based methods for peptide target identification and initiate studies to identify new viral replication inhibitors and their physiological targets using intracellular libraries as tools for chemical genetics in the HCV replicon (aim 4). The integrated technology that will emerge from the work described in this proposal will provide a powerful new tool for the functional dissection of metabolic and signaling pathways in the physiological setting.

Shain, William   Wadsworth Center   Brain Prostheses: Promoting High Neuron Connectivity   NS044287   5 years   NINDS

Abstract: Neural prosthetic devices offer great promise for treating human disease and providing methods for the long-term study of brain neurons. One of our long-range goals is improving the function of neural prosthetic devices by controlling cellular responses around these devices. The primary focus of this proposal is the promotion of high connectivity between neurons and electrodes on prosthetic devices. Such connectivity will replace lost neurons in degenerative disease, e.g. Parkinson's disease, or control pathologic events, e.g. epilepsy, or provide necessary brain input/output following brain injury, e.g. stroke or trauma. We propose to use a multidisciplinary approach to design and fabricate devices that will allow us to test our principal hypothesis that neurotrophic factors, e.g. nerve growth factor (NGF) or brain-derived neurotrophic factor (BDNF) can be used to promote neuron sprouting and direct process growth to electrode sites on prosthetic devices. Principles of chemical engineering and nanofabrication will be used to fabricate micro-machined devices coupled to slow-release polymer matrix materials or fabricated with microfluidic channels to control release of growth factors directly into neocortex. Quantitative methods will be used to describe time- and dose-dependent gradients of neurotrophins. Neuroscientists will assess neuron responses by describing and correlating morphological and electrophysiological measurements. Histochemical methods, direct neuron filling, and image analysis techniques to describe directed growth of neuron processes and their positions relative to electrode and growth factor release sites on prosthetic devices. Measurements of total electrical activity, single unit analysis, and stimulation studies will be used to describe the effects of neurotrophin treatment on connectivity between device electrodes and target neurons. Results from these experiments will provide fabrication strategies to design a new generation of functionally dynamic micro-machined prosthetic devices. These devices will provide highly conductive, long-term functional connections to neurons, insuring their use in chronic treatment and studies of the brain.

Sheetz, Michael P   Columbia Univ New York Morningside   Mechanical Transduction By Cytoskeletons: Proteomic Map   EB001480   5 years   NIBIB

Abstract: ECM composition and forces are major factors in defining the morphology, expression patterns and the structural integrity of tissues. Cells develop and respond to the forces on extracellular matrix (ECM) fibers because those forces are critical to many aspects of tissue and organ function. In engineering functional tissues, it is critical to understand the effects of matrix stretch or relaxation on signaling and other cell functions. The pathways of response to the stretching forces or oscillations in force are poorly understood, but both ion movements and cytoskeletal changes in response to stretch were reported. Recently, we showed that the cellular responses to stretch are observed in Triton X-100 cytoskeletons of cells attached to collagen. In particular, cytoskeletons reversibly bind cytoplasmic focal contact proteins in response to stretch, analogous to in vivo binding. In addition, relaxation of those cytoskeletons causes other cytoplasmic proteins to bind. Normally, the cell assembles an integrated cytoskeleton as it generates a resting tension (traction forces) on extracellular matrix. Substrate or ECM stretching transmits high stresses to the cytoskeleton, which results in cytoplasmic protein binding to matrix contact sites. One major pathway for binding is the stress-dependent alteration of cytoskeletal proteins. Recent results show that a large number of cytoplasmic proteins bind to cytoskeletons in response to stress changes and we propose now to develop a map of the different binding proteins and the signaling pathways to which they are correlated. In order to create this map, we will first develop methods for rapidly identifying the cytoplasmic proteins that bind to cytoskeletons in response to stretch or relaxation on different matrices. Proteomic methods, 2-D gels, mass spectrometric and Western blot analyses, will be utilized to identify the many cytoplasmic proteins involved. We will then study the signaling that occurs in vivo in response to stretch or relaxation as a function of the matrix to which the cell is bound. By comparing and contrasting the stretch- and relaxation-dependent behavior of Triton cytoskeletons from cells on different substrata, we will gain insights into the mechanisms of cell response to force as well as a quantitative measure of the components in the response pathways. These studies will provide a quantitative description of the force-dependent response pathways that form the basis of in vivo and in vitro organ development, wound healing, adaptive changes and certain cancers.

Smolek, Michael K   Louisiana State Univ HSC New Orleans   Computer-Aided Interpretation Of Oculometric Data   EY014162   3 years   NEI

Abstract: The primary goal of the proposed research program is to develop computer software tools with embedded artificial intelligence (AI) that can perform instantaneous, automated analysis and clinical interpretation of wavefront error measurements of the human eye and cornea. Secondary goals are to improve the overall design of oculometric data visualization tools, provide information that will help to establish clinical and scientific standards for ocular measurements and procedures, and improve our understanding of the fundamental relationship between optical performance and visual performance. We hypothesize that a) AI-based algorithms will detect complex patterns of wavefront errors; b) these patterns are specific to and significantly correlated with certain diseases and disorders; and c) AI-based interpretation of complex data will be superior to that performed by expert humans, who are the gold standard for interpreting clinical data. Specifically, we will (1) develop, train, and test AI-based algorithms (Bayesian and neural networks) to interpret the significance of complex wavefront error data obtained retrospectively from examination records of patients with various ocular diseases, disorders, or surgical interventions, as well as normal eyes; (2) simulate wavefront error data using computer models based on statistical distributions of actual ocular aberrations from patient population samples for the purpose of investigating the importance of individual higher order aberrations to retinal image formation and potential visual performance, as well as to generate new data that will enhance the overall AI training and testing process, and (3) establish standard methods to acquire and analyze wavefront error data. AI-based tools will assist vision scientists to efficiently develop study databases and analyze aberration data. Clinicians will diagnose patients faster, more accurately, and with a greater degree of confidence. For patients, refractive surgery outcomes will be more predictable, and they will benefit from earlier detection of diseases such as cataracts and corneal ectasias.

Sporn, Peter H   Northwestern University   Mechanotransduction And Eosinophil Function   HL072891   4 years   NHLBI

Abstract: Eosinophils, the predominant inflammatory cells in asthma, produce large amounts of the 5-lipoxygenase-derived eicosanoid, leukotriene (LT)C4. LTC4 and its derivatives, LTD4 and LTE4, are powerful bronchoconstrictors and potent mediators of asthmatic airway inflammation. In asthma, eosinophils infiltrating the airway wall and intraluminal eosinophils adherent to the airway epithelium undergo cyclic mechanical stretch as the airways distend and elongate with lung inflation during ventilation. To explore whether this dynamic mechanical environment might influence airway inflammation in asthma, we have investigated the effect of cyclic strain on leukotriene synthesis by adherent human eosinophils in vitro. We have observed that adherent eosinophils subjected to cyclic strain, as compared to culture under static conditions, exhibit marked inhibition of LTC4 synthesis in response to agonist stimulation. Preliminary data indicate that this inhibitory effect requires an intact actin cytoskeleton and depends on reactive oxygen species generated in response to cyclic strain, leading to reduced phospholipase-mediated release of arachidonic acid and 5-lipoxygenase enzyme activity. Thus, we hypothesize that cyclic mechanical stretch of adherent eosinophils triggers Rho- and cytoskeleton-dependent signaling, leading to generation of reactive oxygen species. These phenomena result in inactivation of cytosolic phospholipase A2 and 5-lipoxygenase in eosinophils, causing inhibition of cysteinyl leukotriene synthesis. By elucidating the mechanisms by which cyclic strain inhibits leukotriene synthesis in eosinophils, the proposed investigation will provide new information about a major potential determinant of asthmatic airway inflammation that has not previously been recognized. To better understand the effects of mechanical stress on cells in the airway, we have also developed a novel tissue-engineered, 3-dimensional model of the airway wall in which epithelial cells are differentiated at an air-liquid interface overlying fibroblasts in a collagen gel. The gel and cells in the model can be mechanically compressed. We will add eosinophils to the airway model to determine the effects of compressive mechanical stress in the presence of epithelial ceils and fibroblasts on eosinophil leukotriene synthesis.

Stetten, George D   University Of Pittsburgh At Pittsburgh   Tomographic Reflection For Image Guided Intervention   EB000860   4 years   NIBIB

Abstract: We have developed a new tool for guiding invasive procedures with ultrasound, based on a very simple idea. We attach a half-silvered mirror and a small flat-panel monitor to an ultrasound transducer. This resulting device, which we call the sonic flashlight, can project a virtual image of an ultrasound scan into its proper visual location within the patient, without requiring any tracking or head-mounted apparatus. The sonic flashlight provides an intuitive merger of the visual exterior of the patient with the ultrasound image in situ, in vivo, and in real time. It permits the operator to guide a needle through the skin aiming directly at the ultrasound image, using natural hand-eye coordination rather than looking away from the patient at a screen. We believe it will increase accuracy, safety, and speed for a wide variety of diagnostic and invasive procedures, and enable them to be performed with less training. Our ultimate goal is to bring the sonic flashlight to clinical practice. Towards this end we propose to improve the present design in significant ways and to test the sonic flashlight on phantoms and animals, establishing the limits of accuracy and gaining a greater understanding of the human skills involved. Specific Aims: (1) Improve the design of the present 2D sonic flashlight, including its size, weight, ultrasound quality, Doppler capability, mirror optics, ergonomics, etc. (2) Develop a matrix-array 3D sonic flashlight, incorporating a Real-Time 3D (RT3D) ultrasound scanner that will permit the sonic flashlight to display real-time in situ slices with any orientation relative to the transducer. (3)Test human performance using the sonic flashlight on specially developed phantoms, with physical exteriors and simulated ultrasound interiors containing a variety of targets. A mock-up of the sonic flashlight and a needle will be tracked to test human performance in terms of specific psychological processes. (4) Validate feasibility and repeatability of specific image-guided procedures. Then perform statistical study comparing performance of the sonic flashlight to conventional methods. Clinical consultants will be involved at every stage, from studying and redesigning the sonic flashlight to conducting animal trials. If successful, at the completion of the proposed four years of research the sonic flashlight will be ready for testing in humans to guide invasive procedures.

Stetten, George D   University Of Pittsburgh At Pittsburgh   Guiding Vascular Access With The Sonic Flashlight   HL074285   3 years   NHLBI

Abstract: We have developed a new device for guiding invasive procedures with ultrasound, which we call the sonic flashlight. We attach a half-silvered mirror and a small flat-panel monitor directly to an ultrasound transducer to project a virtual image of the ultrasound scan into its actual location within the patient. This permits the operator to guide a needle through the skin by aiming directly at the image, using natural handeye coordination rather than looking away from the patient at a conventional display. The device requires no tracking or head-mounted apparatus, and provides an intuitive merger of the visual exterior of the patient with an in situ ultrasound image, which can be simultaneously viewed by others assisting the operator. We believe the sonic flashlight will increase accuracy, safety, and speed, for a wide variety of invasive procedures, and will require less extensive training. We have narrowed our focus to a single application: the placement of the Peripherally Inserted Central Catheter (PICC) line. The PICC line is increasingly viewed as a safe alternative to direct central line placement in the jugular, subclavian, and femoral veins, while being easier to maintain than a peripheral intravenous line. PICC lines can be placed in the upper arm in the Interventional Radiology suite using ultrasound guidance, or at the patient's bedside, where nurses are beginning to use portable ultrasound. However, success rates at the bedside remain relatively low. We believe the sonic flashlight is well suited for improving this success rate. Our specific aims are as follows: (1) Improve the present sonic flashlight to make it ready for clinical use, (2) Test human performance using the sonic flashlight on phantoms and developing in-vitro training procedures, (3) Validate placing PICC lines by interventional radiologists in patients with the sonic flashlight in the Interventional Radiology suite, and (4) Compare success rates between the sonic flashlight and conventional ultrasound for placing PICC lines in patients by IV nurses in an extended study. If successful, at the completion of the proposed three years, the sonic flashlight will have proven beneficial for the insertion of PICC lines in the hands of skilled IV nurses or other non-physicians. Pending FDA approval (for which the data collected in this study will serve), the sonic flashlight will be ready for routine use at the bedside in the hospital, and other settings.

Stokes, Ian A   University Of Vermont & St Agric College   An Integrated Model Of Intervertebral Disc Function   AR049370   4 years   NIAMS

Abstract: The intervertebral disc is the largest avascular structure in the human body, with very low cellularity and largely without sensory innervation. It has a slow rate of tissue turnover and its viability depends primarily on transport of dissolved nutrients and metabolites, through long diffusion pathways. The disc has been implicated in painful conditions that affect a large proportion of the population. Epidemiological data suggest that long-term degenerative changes are more likely to produce these painful conditions than are acute overload injuries. Therefore, here we propose to combine existing and new information on disc tissue properties and structure into a coordinated theory of the whole disc behavior that can subsequently be applied to explain its healthy and pathological behavior. We will perform experiments to document intervertebral disc mechanical behavior, and compare these data with a new combined theory of mechanical, fluid, and chemical behavior of the disc's tissues and structure. This theory will be incorporated in a finite element model of the whole disc that will be refined based on the experimental data. Specifically: (1) Mechanical behavior will be documented by measurements of time-dependent load-displacement behavior of intervertebral discs and internal displacements. (2) Intervertebral disc swelling behavior will be documented over time by placing semi-constrained discs in baths of differing ionic concentrations and measuring volumetric increase, constraining force and intradiscal pressure. (3) Fluid flow and diffusion in the intervertebral disc will be measured under conditions of different end-plate permeability by recording the concentration of fluorescent dyes of different molecular weights. (4) Electrical potentials will be mapped at known positions in intervertebral discs subjected to time-varying displacements of the specimen with controlled boundary conditions (displacements and porosity of the end fittings). This theory will, in turn, provide a way to understand the relationships between the physical environment of the disc (mechanical, nutritional, etc.) and the local conditions that can influence its metabolism, eventual composition and function in three-dimensions.

Triolo, Ronald J Case Western Reserve University Neuroprosthesis Performance - Nerve Cuff Electrodes EB001889 3 years NIBIB

Abstract: The primary objective of this investigation is to address the limitations of currently available first generation functional electrical stimulation (FES) systems for standing after spinal cord injury by a) activating a greater portion of the targeted muscles to increase available knee extension moment and b) selectively recruiting synergistic muscles to offset fatigue. We will accomplish this through the innovative application of nerve-based cuff electrodes in a series of translational research studies designed to build upon existing animal work and safely and efficiently introduce them into human clinical trials. All current implanted FES systems for standing utilize muscle-based stimulating electrodes that only partially activate the available motor unit pool. While more than adequate for smaller and lighter implant recipients, this approach yields insufficient knee extension moment for heavier or taller candidates. Such individuals require more complete activation of the quadriceps to achieve acceptable functional standing, while simultaneously avoiding the counterproductive hip flexion caused by the femorally innervated sartorius and rectus femoris. The first goal of this study is to demonstrate the feasibility of utilizing stimulating nerve cuff electrodes in standing neuroprostheses, and thus extend the potential user population to individuals who currently cannot take advantage of the technology due to their size and weight. The proximal femoral nerve trunk is composed of numerous fascicles serving structures both advantageous and counterproductive to stable upright standing. Animal studies have demonstrated that a stimulating nerve cuff placing multiple contacts around the nerve can selectively activate individual fascicles within the nerve. The second goal of this investigation is to generate a realistic model of cuff-nerve geometry and determine the fascicular selectivity of multi-contact cuff electrodes on the multi-fascicular human femoral nerve via computer simulation analyses. This will result in an optimized cuff design that maximizes selectivity without detailed a prior knowledge, and thus suitable for clinical use. The third and final goal of this project is to establish the acute and chronic performance of multi-contact cuff electrodes in vivo in human volunteers. Intermittent and cyclic stimulation to individual contacts of chronically implanted electrodes on the distal peripheral nerve branches innervating the vastus lateralis and intermedius will allow fibers to rest while maintaining a constant net submaximal joint moment, effectively increasing duty cycle and allowing some recovery from fatigue. Selectivity of multi-contact nerve cuff electrodes on the proximal femoral nerve will be established in a series of acute intra-operative tests. Completion of this project will extend the functionality of existing neuroprostheses and provide immediate benefit to current system users. It will expand the potential user population, improve consistency of standing performance across individuals, and delay the effects of fatigue. Selective activation of individual muscles from a single multi-contact cuff electrode around a multi-fascicular nerve trunk will simplify the surgical installation of systems that provide more advanced functions such as stepping and stair climbing. Thus, in addition to their immediate impact on the functionality and performance of standing systems, the proposed studies will build a foundation for future developments in lower extremity neuroprostheses by selectively activating the appropriate fascicles in the proximal femoral nerve trunk.

Tsekos, Nikolaos V   Washington University   Methods For MR Guided Interventions In Coronary Vessels   HL067924   4 years   NHLBI

Abstract: Methods for MRI Guided Interventions in Coronary Vessels. MRI can provide the means to assess both vascular and soft tissue morphology and function. For this reason, it is possible to combine vascular interventional MR methods with MR diagnostic methods to provide a single-modality comprehensive test. This combined test would not only diagnose disease, but would also guide interventional procedures and assess the results of intervention. Such capabilities could facilitate new therapeutic approaches and may result in improved management of patients with vascular disease, including coronary artery disease. The objectives of this proposal are first, to develop and implement MRI techniques suitable for MR-guided coronary interventions, and second, to assess the feasibility of performing MR-guided interventions in the coronary arteries. The central principle of the proposed approach is the generation of vascular contrast enhancement by intracoronary infusion of MR contrast agent. The objectives of this proposal will be addressed by performing computer simulations, phantom studies and in vivo studies on instrumented canines. We will pursue four specific aims. (a) Develop and optimize MR techniques, that include pulse sequences and RF coils, for dynamic near-real time multislice imaging of the coronary arteries, based on contrast-enhanced vessel by intracoronary infusion of MR contrast agent, saturation of the underlying tissue and reduction of the field-of-view with outer volume suppression. (b) Establish low-dose MR contrast agent intracoronary infusion protocols, i.e. concentration, volume, and duration of infusion, suitable for multiple dynamic coronary MR-angiography sessions during the same procedure. (c) Develop passive visualization approaches to image and differentiate contrast agent-filled interventional catheters from contrast enhanced coronary vessels, for guiding intracoronary procedures with MRI. Dual lumen and dual-coaxial percutaneous transluminal coronary angioplasty (PTCA) balloon catheter assemblies will be investigated for that purpose. (d)Assess the feasibility of performing MR-guided balloon PTCA on a canine model of graded generated stenosis to assess the results of the intervention via contrast-enhanced first pass perfusion imaging of the myocardium distal to the stenosis.

Turnbull, Daniel H   New York University School Of Medicine   Ultrasound And MR Imaging Of Mouse Brain Development   NS038461   5 years   NINDS

Abstract: The availability of transgenic and gene targeting methods, and the large number of spontaneous and induced mutant mice with altered neural development, have led to significant progress in dissecting the genetic pathways involved in patterning and cell specification in the mouse nervous system, and in producing many mouse models of human neurodegenerative diseases. Despite these advances, little is known currently about the relationship between genetic changes and altered brain function, in part because of the lack of readily available and efficient quantitative methods to analyze functional changes in the mouse brain. We have developed both ultrasound biomicroscopy (UBM) and magnetic resonance micro-imaging (muMRI) approaches to analyze anatomical and functional neural development and disease in the mouse from early embryonic to adult stages. UBM-guided injections have also provided the means to perform rapid, direct gain-of-function genetic studies in the embryonic mouse CNS. The combination of UBM and muMRI approaches being developed can provide in vivo assessment of brain function in normal and disease model mice. The broad goals of this project are to develop in vivo micro-imaging approaches enabling analysis of normal and abnormal function in the developing mouse brain, and the relationships between genetic changes and altered brain function from embryonic to adult stages. The specific aims are: 1) To develop and test two new direct methods for gain-of-function studies in the embryonic mouse CNS. 2) To test whether transgenic, CNS-specific over-expression of iron transport and storage proteins can be used for in vivo imaging of gene expression with gMRI. 3) To develop UBM and pMRI methods to quantify blood volume and flow in the developing mouse brain. 4) To quantify changes in manganese-enhanced MRI resulting from stimulation of neuronal activity in the early postnatal to adult mouse brain. The combination of genetic engineering approaches in the mouse with advanced ultrasound and magnetic resonance micro-imaging technology will provide powerful new tools for analyzing mouse developmental neurobiology, and leading to new insights into mammalian brain development and neurodegenerative diseases.

Udelson, Daniel G   Boston University Charles River Campus   Expandibility As A Measure Of Fibrosis Induced Impotence   AG019699   3 years   NIA

Abstract: Erectile dysfunction is common, developing in 52% of men aged 40-70 years, and the host significant physiological correlate is increasing age. Independent of vascular disease, erectile tissue undergoes progressive loss of corporal trabacular smooth muscle and increase of corporal connective tissue. If clinicians were able to accurately measure in a non-invasive manner the degree of corporal fibrosis, they would be in a position to develop management strategies to prevent or delay the onset of erectile dysfunction. The clinical utility of an assessment of corpus cavernosum fibrosis is primarily early detection of initial structural disease, a critical component of erectile dysfunction prophylaxis. The broad, long-term objective of this engineering-based medical research is the development of a clinically-applicable method to assess corpus cavernosum fibrosis. The specific aims to achieve this goal will be to: (i) develop a refined engineering model that illustrates the relationship between cavernosal percent smooth muscle and cavernosal "expandibility". (2) demonstrate, in a group of patients, correlation between cavernosal "expandibility" (obtained during pre-operative dynamic infusion pharmacocavernosometry) and cavernosal percent smooth muscle (obtained by histomorphometric analysis of the cavernosal tissue harvested during penile implant surgery). This would verify that the correlation between these two parameters, which was shown to exist in a previous animal study, also exists in humans; and (3) clinically test a circular strain gauge device that automatically measures penile geometry versus intracavernosal pressure which is necessary for the calculation of cavernosal "expandibility". It is anticipated that this project will contribute substantially to a new clinical assessment of the structural effects of aging on erectile dysfunction which is necessary to develop rational effective clinical strategies to offset the age-related decline in erectile function.

Utzinger, Urs   University Of Arizona   Optical Spectroscopy Of Ovarian Cancer   CA098341   3 years   NCI

Abstract: The objective of this work is to determine the etiology of endogenous optical signals from ovarian tissue. This research will serve as the basis for development of a minimally invasive method for the diagnosis of pre-malignant changes as well as early ovarian cancer using fluorescence and reflectance spectroscopy. The hypothesis that drives the proposed research is that developing a series of experimental and mathematical models will allow us to explain the differences in optical signatures of normal ovaries, premalignant changes, and malignant transformations. By understanding this contrast, we will be able to derive effective early diagnostic methods for ovarian cancer and improve early detection of this highly fatal disease. Diagnostic techniques will be most useful in women at high risk of developing ovarian cancer to identify those women who need to undergo an oophorectomy. Once a serum based screening test is available for the low risk population it will be of utmost importance to perform a second lock diagnostic procedure because even excellent tests will generate a large number of false positive results. We propose the four following specific sub-projects: 1) collect spectral data of cellular and extracellular constituents of normal and transformed ovarian tissue; 2) characterize optical tissue signals in vivo and obtain biopsies from the same interrogated tissue volume; 3) use these biopsies to study etiology of the optical signals in an in vitro tissue culture model; and 4) synthesize mathematical models of remitted optical signals based on all collected data to explain the biophysical sources of spectral variations and to develop novel diagnostic metrics. The projects will advance present knowledge of the development of cancer, a health problem which, notwithstanding significant medical advances over the past fifty years, remains the second leading cause of death in the United States as we enter the new millennium. The proposed project will take an important step toward improving the overall survival in ovarian cancer, a statistic that has not changed in the last 50 years.

Vernon, Brent L Arizona State University Polymers With Time-Dependent Properties For Drug Deliver GM065917 5 years NIGMS

Abstract: The overall goal of this research is to develop a new class of in situ-forrning, injectable, and biodegradable polymeric biomaterials based on time-dependent molar mass and lower critical solution temperature (LCST) properties for localized delivery of an anti-cancer agent, phenstatin. Many current biodegradable, injectable, and in situ-forming biomaterials under development have disadvantages including the use of water miscible organic solvents for delivery, low molecular weight toxic byproducts, reactive chemistries and the need for external light sources (i.e., for photopolymerization). Ideal replacement materials for these applications would be easily injected and form in a timely fashion without detrimental effects to surrounding tissue from temperature increases, toxicity or invasive techniques. Many of these difficulties can be addressed using NIPAAm copolymers with time-dependent molar mass and lower critical solution temperature (LCST) properties. Copolymers of N-isopropylacrylamide (NIPAAm), methacrylic anhydride(MA) and maleic anhydride (MAn) will possess time-dependent LCST properties in an aqueous environment due to the conversion of maleic anhydride side chains to maleic acid and time-dependent molar mass due to conversion of methacrylic anhydride to methacrylic acid, both by hydrolysis. Copolymers of NIPAAm, methacrylic anhydride, and maleic anhydride will be synthesized and be characterized for initial and final LCST, initial and final molar mass, the initial strength of the gel, and degradation time. Drug release profiles will be evaluated from selected materials. The cytotoxicity and biocompatibility of these materials will be assessed using cell proliferation (MTT) and live/dead assays on BALB/c 3T3 cells. Tissue irritation potential of these materials will be evaluated in vitro by macrophage and lymphocyte activation experiments. Finally, a selected material will be injected subcutaneously into Sprague Dawley rats to verify injectability and in situ formation. In vivo compatibility will be assessed using selected histological techniques. In vivo efficacy of phenstatin released from this material will be evaluated using a SCID mouse ovarian cancer model.

Voytik-Harbin, Sherry L Purdue University West Lafayette Cell-ECM Interactions: A 3d Micro-Mechanical Perspective EB000165 4 years NIBIB

Abstract: The long-range goal of the research is to foster development of better approaches to overcome the devastating problems of tissue loss and organ failure. The objective of this proposal is to determine how specific micro-structural and mechanical properties of a 3D extracellular matrix (ECM) define the distribution and transfer of mechanical signals to cells that in turn regulate their response and ultimately contribute to the overall tissue structure/function. The central hypothesis for the research is that the cellular response to micro-structural composition and micro-mechanical loading within a 3D ECM is mediated, in part, by the distribution and composition of cell-matrix adhesions. The rationale for the research is that definition of critical 3D structural and mechanical features of a cell's ECM environment, especially at the micro level, as well as identification of the mechanisms by which they influence cell behavior will make it possible to engineer biomaterials with specific material properties that predictably induce a cellular response that accelerates or improves tissue restoration. The objective of the proposed research will be achieved by pursuing three specific aims: 1) determine the morphological, phenotypic, cell-matrix adhesion, and ECM remodeling properties of fibroblasts within 3D ECMs in which the micro-structure is quantified and controllably varied and no external mechanical loads are applied; 2) determine the morphological, phenotypic, cell-matrix adhesion, and ECM remodeling properties of fibroblasts within 3D ECMs in which the micro-mechanical properties (e.g., 3D micro level stress and strain fields) are quantified and controllably varied by application of external mechanical loads; and 3) identify mechanisms by which fibroblasts perceive the micro-structural composition and micro-mechanical state of the surrounding 3D ECM. Expertise in the areas of ECM biochemistry, cell biology, biomechanics, and bioimaging will be combined to yield the following outcomes. First, specific structural-mechanical attributes of a cell's ECM micro-environment will be quantitatively defined. In turn, the dependence of the cellular response on specific micro-structural and mechanical properties of a 3D ECM will be established in the presence and absence of externally applied mechanical loads. Third, the effect of other major cellular signals on the ability of cells to sense and response to these biophysical cues will be determined. Fourth, key events associated with celI-ECM adhesion that provide cells with the ability to sense and respond to physical properties of a 3D ECM microenvironment will be identified. Collectively, these outcomes will provide new information regarding the physical aspects of celI-ECM interaction and establish the role of cell-matrix adhesions in the ability of cells to respond to 3D structural and mechanical cues provided by the ECM. This research is significant to tissue engineering and medicine because the results are expected to define much needed fundamental principles and design criteria that will lay the foundation for directed repair of damaged tissues.

Wang, Lihong   Texas Engineering Experiment Station   Full Polarization Characterization By OCT   CA092415   4 years   NCI

Abstract: The long-term objective of the proposed research is to develop a novel, non-invasive tool for microscopic imaging of superficial lesions, including cancers and burns. The short-term goal is to apply the proposed technology in small-animal imaging. The proposed technique, Mueller-matrix optical coherence tomograph, (OCT), can image in real time the complete polarization properties of intact biological tissues for the first time at the microscopic scale (about 10 microns) in vivo. Optical polarization properties are sensitive indicators of physiological states such as the collagen content and abnormalities of biological tissues such as necrosis, and they can provide novel contrast mechanisms for imaging. Initially, this technology will likely have an impact on small-animal experimental studies because it can reduce the number of animals needed and the time required to conduct a study and can also improve the temporal correlation of a study. In addition to the immediate applications, this technology has the potential to detect various superficial human diseases--such as oral, skin, cervical, colon, and bladder cancers as well as skin burns--that can be accessed either directly or endoscopically as already demonstrated by conventional OCT. Mueller matrices can completely characterize the polarization properties of any material. The applicants' group pioneered Mueller-matrix OCT and demonstrated that this new imaging modality reveals tissue structures that are not observable with conventional OCT. Striking polarization contrast has already been shown in burns by Mueller-matrix OCT. The specific aims of the proposed research are as follows, in which the animal experiments will have dual foci: the imaging of skin cancers (animal model 1) and the imaging of burns (animal model 2). Aim 1. Develop a free-space Mueller-matrix OCT system to image Mueller matrices of biological tissues with both depth and lateral resolutions in real time. Aim 2. Characterize the capability of the proposed system and understand the origin of OCT polarization contrast by imaging tissue samples ex vivo. Compare the Mueller-matrix images with the corresponding histological results from both conventional and polarization light microscopes and identify the relationships between the Mueller-matrix images and the histological structures. Aim 3. Further develop the free-space Mueller-matrix OCT system using fiber optics and construct a hand-held probe for in vivo applications. Compare the experimental results from the fiber-optic system with those from the original free-space system. Aim 4. Characterize the capability of the proposed hand-held probe by imaging skin cancers in vivo in a mouse model (animal model 1). Detect the location, size, and Mueller-matrix contrast of skin cancers in comparison with the histological results from both conventional and polarization light microscopes. Analyze and characterize the temporal progression of skin cancer in the animal model. Aim 5. Characterize the capability of the proposed hand-held probe by imaging skin burns in vivo in a mini-pig model (animal model 2). Detect the lateral extent, depth, and Mueller-matrix contrast of skin burns in comparison with the histological results from both conventional and polarization light microscopes. Analyze and characterize the temporal healing process of burns in the animal model.

Webb, Andrew G   University Of Illinois Urbana-Champaign   Cerebral Hemodynamics By Simultaneous Function MRI   MH065429   3 years   NIMH

Abstract: Studies of the kinetics of cerebral metabolism and hemodynamics require fast, non-invasive neuroimaging techniques with high biochemical specificity. Although functional magnetic resonance imaging (fMRI) techniques are fast, noninvasive, and have relatively high spatial resolution, they do not provide the biochemical specificity needed to characterize the behavior of important physiological parameters such as oxy- and deoxyhemoglobin concentrations. Near-infrared spectroscopy (NIRS), however, does have the biochemical specificity necessary to measure hemodynamic changes, in terms of the concentrations of both oxy- and deoxyhemoglobin, but is typically characterized by relatively poor spatial resolution. To explore the advantages of using NIRS together with fMRI we propose the study of dynamic aspects of cerebral blood flow and metabolism by designing sensors able to perform high sensitivity simultaneous NIRS and fMRI experiments. We will use fMRI methods to determine the location of hemodynamic change and NIRS data to uncouple temporal changes in oxy- and deoxyhemoglobin and to determine the spatial location of these latter changes. As a result we expect to develop more realistic biophysical models of functional cerebral perfusion and oxidative metabolism, and to understand more fully the physiological mechanisms of the blood-oxygen-level-dependent (BOLD) effect used in fMRI. A broader impact of this project will be the further development of low-cost, noninvasive, and fast near-infrared techniques for neuroimaging. This method will allow measurement of local cerebral oxygenation and hemodynamic changes in humans and will be fully compatible with MRI instrumentation.

Webb, Robert H   Schepens Eye Research Institute   Illumination/Display Technologies In Vision Research   EY014165   3 years   NEI

Abstract: This is a proposal based in newly available technologies to allow simple implementations of psychophysical and imaging procedures that have been known but difficult in the past. New ultra-bright LEDs (light-emitting diodes) now make it possible to present high brightness displays without laser speckle. Spatial light modulators (SLMs), either liquid crystal or MEMs mirror based, allow these displays to have the necessary high contrast. The resulting displays make possible Maxwellian view psychophysics at very high speeds, extended intensity range and high linearity. The same technologies, applied to live imaging of the anterior ocular components (lens, cornea, vitreous) will allow imaging using confocal techniques without lasers. Finally, with the newly accessible wavelengths, we can tailor the imaging to give a pseudo-color image that is really in the near infrared, but that can be directly related to the familiar visible colored images. This proposal therefore addresses three apparently quite different areas of ophthalmic investigation, linked by the technologies that will enable them.

Yoon, Guenyoung   University Of Rochester   Customized Contact Lenses   EY014999   4 years   NEI

Abstract: Wavefront aberrations in the eye's optics degrade vision. These aberrations, including many higher order aberrations that are not corrected by conventional spectacles, are especially severe in patients with keratoconus and in patients who have had penetrating keratoplasty. Accurate measurement and correction of these higher order aberrations could result in substantial improvements in vision. However, little wave aberration data can be obtained from patients with these conditions, primarily because existing wavefront sensors have too small a dynamic range to measure the large aberrations in these eyes. Moreover, even if measurements were available, there are few available therapeutic alternatives. The research objectives of this bioengineering research project are to develop a robust wavefront sensor, with a large dynamic range, that will reliably diagnose the wave aberrations in highly aberrated eyes, and to develop a customized contact lens that can compensate for most of these aberrations. The key to expanding the dynamic range of the wavefront sensor is the use of a translational plate that increases spacing between wavefront sensing spots. The key to developing the contact lens is the use of high-power laser ablation of the contact lens based on the measurements with the wavefront sensor.