PI Name	Institution	Title	Project	Years awarded	Funding Institute
Andersen, Richard A	California Institute Of Technology	Neural Prosthesis Using Posterior Parietal Reach Region	EY013337	5 years	NEI

Abstract: DESCRIPTION:(adapted from applicant's abstract) The purpose of this grant is to develop a neural prosthesis to help paralyzed patients. The prosthesis will be developed in non-human primates as a precursor to applying a similar approach in humans. The rationale of the prosthesis is to record from an area of the cerebral cortex that plans reach movements. If these plans can be read-out in real-time, then patients who are paralyzed from spinal cord section, ALS, or other peripheral neuropathies could still think about making movements, and these thoughts could be used to operate external devices. In the experiments, the activity from the parietal reach region (PRR) will be recorded using arrays of electrodes, an area that is responsible for the initial planning of reach movements. Decode algorithms will be developed that will allow these plans to be read out in real time. The output device will be a robot limb whose controller is designed to be instructed by high level signals and to compute many of the lower level aspects of the movement trajectory that are normally computed at levels of the brain closer to the motor output. This hybrid control system represents a new area of robotics research. Additionally, local field potentials will be used to convey similar information to the robotic controller and this should provide a breakthrough for long-term recordings. The specific aims will proceed from the simplest experiment of demonstrating that a monkey can control an animated limb on a computer screen to more complex experiments where the experimenters will determine how PRR codes information about reaches in more natural situations. These later experiments will include neurophysiological studies of the coding of sequential movements, curved trajectories, combined hand-eye movements, and visua-motor plasticity. The concurrent engineering studies will develop alogorithms that can reconstruct the intended movements from neural record and develop supervisory control architectures that can move the robotic limb appropriately, all in real-time.

Barron, Annelise E	Northwestern University	Development of a Biomimetic Lung Surfactant Replacement	HL067984	4 years	NHLBI

Abstract: We propose to develop a novel class of biomaterials called "polypeptoids," or poly-N-substituted glycines, and to apply them to a specific biomedical problem: the need for more effective synthetic, functional mimics of the human lung surfactant proteins SP-B and SP-C. Lung surfactant (LS) is a surface-active material that coats the internal surfaces of healthy mammalian lungs and enables breathing, by reducing the surface tension on the alveolar surfaces. LS is composed of 95 percent surface-active lipids and 5 percent surfactant-specific proteins; both lipid and protein fractions are necessary for its functioning. Two of these surfactant-specific proteins, SP- B, and SP-C, are especially surface-active and are critical for the proper biophysical functioning of LS in vitro and in vivo. SP-B and SP-C are both small, helical, amphipathic proteins (79 and 35 amino acids, respectively); essentially, just peptides. Premature infants born before about 30 weeks of gestation are born with immature lungs lacking surfactant, and require the delivery of an exogenous lung surfactant replacement at birth to enable mechanical ventilation. At present, the most efficacious LS replacement formulations are animal- derived, and therefore raise concerns about their level of purity, their consistency of formulation, and their potential for pathogen transmission, as do any medicines sourced directly from animals. While synthetic LS replacements do exist, they do not work as well as animal- derived surfactant replacements, primarily because these formulations lack good functional replacements for SP-B and SP-C proteins. We propose to develop functional mimics of SP-B and SP-C based on poly-N-substituted glycines, which are sequence- specific heteropolymers synthesized in a similar manner to synthetic polypeptides, by a facile, automated solid-phase protocol. Peptoids offer the advantage s of protease- resistance, biomimetic helical secondary structure, low immunogenicity, and low cost. Peptoid-based SP-mimics will be synthesized, purified, and their secondary structure and biophysical surface activities will be analyzed in vitro circular dichroism spectroscopy and by equilibrium and dynamic surfactometry. The feasibility of these novel SP- mimics is demonstrated in preliminary work. Promising formulations will be tested in vivo by a collaborator.

Boone, John M | University Of California Davis | Feasibility Of CT In High Risk Breast Cancer Patients | CA089260 | 3 years | NCI

Abstract: Mammography is used to screen asymptomatic women for breast cancer, and typical breast cancer found using mammography is approximately 11 mm in diameter. At this small size, removal of lesion results in breast cancer cure in the majority of women. However, there is a small class of women (about 5% of all breast cancers) who are genetically predisposed to breast cancer (BRCA1 and BCRA2 genes), and in these women, more aggressive detection methods are needed. In addition to BRCA1 and BRCA2 carriers who are at extraordinary risk of breast cancer, women with extremely dense breasts are at higher risk from breast cancer (by virtue of their dense breasts with odds ratio from 4 to 6), and mammography is less sensitive in these women. For women in these high-risk categories, most of whom have dense breasts that are poorly imaged by mammography, an imaging modality with better lesion detectability performance (contrast resolution) is needed. While ultrasound rely on contrast mechanisms that are less reliable than X-ray contrast-that is why they are not used for screening. However, computed tomography (CT) does depend upon x-ray contrast mechanisms, but has about 10 times the contrast resolution as projection mammography. CT is very capable of identifying soft tissue lesions in the 3-5mm range- Such lesions are 10 to 50 times smaller in volume than the average 11mm lesion found by mammography. Therefore, CT has great potential for much earlier detection of breast cancer than mammography for high-risk patients. In this feasibility study, we propose to throughly investigate the potential of dedicated breast CT using computer simulation techniques coupled with CT of cadaver breasts and mastectomy specimens. Monte Carlo studies will be used to fully evaluate the glandular dose of breast CT, and imaging studies will be used to define the requirements of optimal CT acquisition. Using CT scans of breast lesions from about 10 mastectomy specimens, a breast tumor model will be developed. The tumor model will be used with a series of about 20 cadaver breast CT data sets to conduct extensive ROC studies. Computer observers will be used to define the Az versus tumor diameter curves for both CT and mammography. Human observers will be used to validate and calibrate the more extensive computer observer results. The results of this investigation should provide a clear understanding of the potential of breast CT as a tool to reduce breast cancer mortality in the population of women with dire risk of breast cancer.

Bottomley, Paul A | Johns Hopkins University | Innovative MRI Research Technology | RR015396 | 5 years | NCRR

Abstract: DESCRIPTION (Provided by Applicant): New advances in magnetic resonance imaging (MRI) show enormous potential for a broad range ot new clinical and research applications including high-speed cardiovascular imaging, functional brain imaging, and contrast-sensitive image-guided intervention. They promise important advances in the assessment and treatment of cardiovascular disease, cancer and brain function. Central to their success are the dual demands of imaging speed and signal-to-noise ratio (SNR). Core biotechnology research that significantly advances MRJ speed and SNR are pivotal to progress and could impact new MRI applications across-the-board. This proposal, responsive to PAR-99-009 for Bioengineering Research, will directly advance MRI speed and SNR with innovative research in fourkey areas: the MRI gradient systems; high-speed multi-channel MRI receivers; new high-speed relaxation time (Ti) measurements and imaging; and the design of MRI detector coil systems that deliver the ultimate intrinsic SNR (UISNR)-the maximum that can be had. Specifically, we propose to develop compact, highly-efficient surface MRI gradient modules that can significantly increase gradient strength by more than an order of magnitude, and reduce gradient rise-times, and to develop algorithms to correct gradient nonlinearity. We will demonstrate the gradient advantages in a pilot study of diffusion MRI in acutestroke. We will develop a high-speed multi-channel receiver system with new analytic reconstruction methods for sensitivity-encoding to achieve manifold reductions in the minimum scan-time, and to realize the speed gains from the other projects, and we will demonstrate this with real-time MRT stress-testing of patients with ischemia. Ti relaxation is key to MRI contrast, and we propose new methods that promise dramatic reductions in Ti measurement times, Ti imaging, and we will demonstrate its potential for temperature monitoring during RF ablation therapy. andin human Ti studies. Finally, we introduced the USINR concept, and will now develop tools for designing coils that yield the UISNR, and build them for the head, heart, and abdomen, and will compare their performance to existingcoils. We hypothesize that the integration of radical redesigns of gradient, MRI detector coils, and high-speed contrast-sensitive MRI, will yield simultaneous performance gains that will provide truly new opportunities for clinical diagnostic and interventional research. The work will be implemented at 1 .5T and will directly and positively benefit 21 other funded research grants at this institution, with broad potential benefit to noninvasive imaging in a wide range of patients and diseases.

Cho, Michael R | University Of Illinois At Chicago | Electromechanical Control Of Cell Adhesion And Motility | GM060741 | 5 years | NIGMS

Abstract: DESCRIPTION (provided by applicant): Tissue engineering is a relatively new but rapidly expanding field of biomedical engineering research. Because cell adhesion and motility are two critical factors in determining tissue integrity and function, elucidation and regulation of the cellular and molecular mechanisms involved in cell adhesion and motility is fundamentally important for tissue engineering. Implementation of engineered tissues in clinical applications has been successful in soft-tissue replacement or regeneration. The general use of artificial tissues is limited, however. Detailed understanding of mechanisms regulating cell adhesion and motility is expected to provide insights for controlled cell seeding, improved designing and engineering of artificial tissues. Use of non-invasive electrical stimulation (ES) offers a novel non-mechanical technique to regulate cell adhesion and motility. Projects involving the use of ES in fibroblasts, hepatocytes, and white cells and studies of lateral and rotational dynamics of membrane proteins provided an excellent basis for the proposed hypotheses in this proposal. In response to ES, the mechanotransducer integrin is likely to mediate cell adhesion and motility. Integrins redistribute on the cell surface, interact with cytoskeleton, and actively participate in dynamic formation of focal adhesion contacts. Optimized use of ES has been shown to redistribute integrins, reorganize cytoskeleton, alter calcium homeostasis, and induce guided cell migration without adversely affecting cell viability. This proposal uses unique non-invasive optical techniques, including single particle tracking and laser optical trap, to 1) track, at the single molecule level, changes in integrin motion induced by ES on the surface of human fibroblasts; 2) measure changes in the strength of integrin-cytoskeleton interactions induced by ES on controlled 2 dimensional extracellular matrices; and 3) characterize electromechanically induced and integrin-dependent cell motility in reconstituted 3 dimensional gel model. The long-term objectives of the proposed research are to manipulate and control cell adhesion and motility by the optimal use of ES and, thereby, to enhance tissue integrity and function of engineered tissues.

Colton, Richard J | U.S. Naval Research Laboratory | Single Cell Detection And Analysis | GM061358 | 3 years | NIGMS

Abstract: DESCRIPTION: (adapted from applicant's abstract) The objective of the research is to demonstrate the feasibility and utility of a new analytical instrument capable of single cell detection and genetic analysis. The principal component of this approach is a micro-array of giant magnetoresistive (GMR) sensors to measure selected binding reactions. The system is referred to as BARC, which stands for bead array counter. The basic process involves labeling a selected analyte with a magnetic bead which is then detected by means of the magnetoresistive effect. The experimental plan is designed to establish proof-of-concept by using a prototype instrument based on an 8x8 array. System attributes will be established with the detection and characterization of a selected strain of yeast. Specific aims are to 1) develop the magnetic labels and assays for yeast cell capture, 2) apply surface patterning techniques to prepare an array of PNA probes, 3) design a prototype unit capable of performing all processing steps, 4) design a disposable cartridge system with integrated microfluidics, and 5) test the overall performance of the final prototype probe.

Constantinidis, Ioannis	Emory University	Effects Of Alginate Composition On Encapsulated Cells	DK056980	1 years	NIDDK

Abstract: DESCRIPTION: (verbatim) Use of alginates to encapsulate cells in order to provide mechanical support and immunoprotection is common practice in the development of a bioartificial pancreas. Alginate is a family of linear binary heteropolymers composed of a-L-guluronic acid and b-D-mannuronic acid residues in various proportion, sequence, and molecular weight. Given their variety in composition, it is our hypothesis that the behavior of encapsulated cells will depend on the composition of the alginate matrix. It is our objective to study the interaction between alginate matrix and encapsulated cells in model tissue-engineered pancreatic constructs. These model constructs are composed of neonatal porcine islets or mouse insulinoma bTC3 cells encapsulated in one of four different types of alginate. The specific aims of the proposed research are to determine the property of alginate gel responsible for affecting the behavior of encapsulated cells, and conversely to investigate the affect of encapsulated cells on the alginate matrix, in vitro and in vivo. To complete the first specific aim, the metabolic and secretory profiles of the encapsulated cells will be monitored, and the apoptotic, proliferative, viable and necrotic cell fractions determined. To complete the second specific aim, 1H NMR techniques will be employed to monitor changes in the alginate matrix. Overall, the proposed experiments are a thorough investigation of the interaction between alginate, as an extracellular matrix, and encapsulated cells. The criteria to judge this interaction are: insulin secretion; bead integrity; cell growth; and in vivo stability. It is anticipated that we will identify alginate compositions that are beneficial to a tissue-engineered pancreas as well as compositions that are inappropriate. Furthermore, the proposed NMR experiments will enhance our understanding of the capabilities of NMR to monitor a tissue-engineered construct in vitro and in vivo.

Darquenne, Chantal J	University Of California San Diego	Modeling of Aerosol Transport in Alveolated Airways	ES011177	3 years	NIEHS

Abstract: The long-term objective of this study is to better understand the fate of inhaled particles in the human lung. Exposure to particulate matter (PM) has been a major concern in the recent years as more evidence links air pollution and mortality. A better understanding of aerosol deposition (DE) in the lung may also be beneficial in applications such as inhalation drug therapy. Mathematical models are often used to help interpret the experimental studies and to make predictions for cases where experimental data are not available. Aerosol transport in both symmetrical and asymmetrical two-dimensional models of the alveolar zone of the human lung will be simulated ro 0.5 to 5 mum particles is mainly due to gravitational sedimentation. The orientation of the structure with respect to the gravity vector is a major factor affecting DE patterns. CM causes inhaled particles to be irreversibly transferred to the resident air during their transport through the lung. Because of this transfer, some particles that do not deposit on the airway walls remain in suspension in the distal airways at the end of a normal expiration and fail to exit the lung. These particles then penetrate deeper in the lung during the next breath and eventually deposit. Velocity profiles in the alveolar region of the lung are expected to be a major factor that affects CM. The simulations will allow us to isolate their effect and to determine their contribution to overall CM. Finally the effects of extra CM caused by stretch and fold on the alveolar DE of particles in the human lung will be simulated. Stretch and fold refers to the process where, because of non-reversibility of flow in the lung, air streamlines become folded back on themselves, enhancing mixing. The results of this study may provide a link to the mechanisms by which even seemingly modest exposure to PM can cause or exacerbate lung disease. The results will also help to better design spatial targeting of drugs administered by inhalation therapy.

Durand, Dominique M | Case Western Reserve University | Activation Of Tongue Muscles In Obstructive Sleep Apnea | HL066267 | 4 years | NHLBI

Abstract: DESCRIPTION:(adapted from applicant's abstract) This project aims to develop an experimental therapy for obstructive sleep apnea (OSA) using electrical stimulation of the hypoglossal nerve. The PI proposes to use a multi-channel stimulation technique for selective activation of portions of the hypoglossal nerve to activate individual tongue muscles selectively. Such stimulation might increase control over the tongue muscles and improve efficiency for electrical stimulation to remove pharyngeal obstruction. There are 3 aims to be tested in dogs. Aim 1 will determine the maximum muscle selectivity that can be obtained with a multi-contact electrode on the hypoglossal nerve. Aim 2 will evaluate the mechanical dilation of the airways due to selective stimulation. Aim 3 will determine the optimum electrode geometries and stimulation paradigms for airway dilation in chronic dogs. It is anticipated that the experimental delineation and validation of these methods will lead to a design of a neuroprosthetic device for the treatment of OSA.

Eppstein, Margaret J | University Of Vermont & St Agric College | 3 D Frequency Domain Optical Mammography with APPRIZE | CA088082 | 3 years | NCI

Abstract: Optical imaging of breast tissues with near infrared (NIR) light has the potential to become an important tool in the diagnosis of breast cancer and the monitoring of deep tissue photodynamic breast cancer therapies. Unlike x-ray mammography, optical techniques are non-ionizing and may provide information about malignant tumors, although the resolution of optical mammograms is complicated by the diffuse propagation of light through tissue. Current academic and commercial approaches to optical mammography have largely focused on 2-D imaging techniques, due to the computationally intensive inverse methods employed. A powerful new method for 3-D optical imaging of breast tissues that dramatically differs from existing approaches is proposed. Recently invented techniques for both data collection and data inversion are integrated and co-optimized for use in a novel 3-D "brassiere cup" geometry that is non-compressive, comfortable, and maximizes signal to noise of the measurements. Frequently domain photon migration data will be collected at the surface of breast-shaped tissue-mimicking phantoms, with and without exogeneously introduced fluorescent contrast agents. Optical property maps for absorption, fluorescent-enhanced absorption, and fluorescence lifetime will be estimated using the rapid Bayesian inversion method known as APPRIZE. APPRIZE, unlike other inversion methods, explicitly accounts for measurement noise and system noise, and yields minimum variance estimates of optical property maps and their uncertainties. The use of innovative data-driven zonation dramatically improves the computational efficiency, stability, and accuracy of APPRIZE, relative to other competing inverse methods, and 3-D optical inversion has already been demonstrated. The continued development of sophisticated domain-decomposition strategies, along with other algorithmic improvements to APPRIZE, is expected to render high-resolution 3-D optical mammography a reality.

Gilbertson, Lars G | University Of Pittsburgh At Pittsburgh | Development Of A Robotics-Based Spine Testing System | AR047336 | 3 years | NIAMS

Abstract: DESCRIPTION (provided by applicant): The prevalence of musculoskeletal +_____ impairments of the spine in the U.S. is estimated to be greater than 18 million-over one-half of all musculoskeletal impairments. Biomechanical testing of osteoligamentous spinal specimens has become fundamental to the investigation of the role of mechanical loads (i.e., forces and moments) in the etiology and management of spinal impairments. There is no consensus of opinion how loads are to be applied to a specimen to best represent in-vivo loading conditions. Current arguments center on whether biomechanical tests of spine are best done in a "load control" mode or a "displacement control" mode. However, evidence suggests that in-vivo, the spine does not operate exclusively in either of these control modes, but rather in a combination of load and displacement control-i.e., "hybrid control.' Existing spine testing systems fundamentally do not have the capacity to operate in hybrid control, therefore posing an obstacle to realistic control/application of experimental loads. The leading edge of hybrid control development and implementation is in robotics-thus the objective of this proposal is to develop a robotics-based spine testing system, and use it to study in-vitro spinal biomechanics under hybrid control. Four specific aims are proposed: (1) Develop and validate a robotics-based spine testing system with programmable hybrid control. (2) Delineate the in-vitro kinetics (i.e., load-displacement response) of human lumbar functional spinal units (FSUs) under hybrid control. (3) Determine the effects of degeneration and (4) surgery on in-situ forces of the disc, ligaments and facet joints. A computer-based robotic controller will be developed and validated using experimental and analytical approaches to enable an existing commercial robot to apply loads to human lumbar FSUs under hybrid control. Overall kinetic response as well as functional role of the structural elements of the specimens will be delineated during external loading of the FSU. Radiographic and morphological analyses will be done to correlate biomechanical properties with degenerative changes. The knowledge gained from this study will improve our understanding of the biomechanics of the spine under control modes more representative of in vivo conditions, and will help to elucidate the role of degenerative changes in the load-bearing of the spine.

Gross, Ted S | University Of Washington | Augmentation of Peak Bone Mass | AR048102 | 4 years | NIAMS

Abstract: One of the primary risk factors associated with osteoporotic fractures is the failure to achieve sufficient bone mass during early adulthood. Exercise holds substantial potential for non-invasive bone accretion, but general exercise protocols only minimally enhance bone mass over normal levels and rely upon high impact, high magnitude loading to achieve these bone gains. Recently, we found that inserting a brief rest interval between each load cycle of a low magnitude loading regimen is sufficient to transform the protocol from one that does not influence bone cell populations into a signal that is potently osteogenic. This regimen may therefore be particularly amenable to application in conditions where enhanced bone properties are desirable, but high impact exercise is not feasible. Based upon our preliminary data and a review of the literature, we hypothesize that low magnitude, rest-inserted, mechanical loading initiated during skeletal growth will enhance the cortical bone properties of adult mammals. To explore this hypothesis, we will capitalize on our recently developed non-invasive murine tibia loading device. A series of five Specific Aims have been designed to determine if rest-inserted, low magnitude loading can serve to build and maintain augmented tibial cortical bone properties in female C57BLI6J mice. The studies culminate with our final Aim, in which we will assess whether enhanced cortical bone properties induced by the rest-inserted loading are sufficient to counteract the degradation caused by aging and estrogen depletion. At a basic level, these studies will provide unique insight toward how young growing bones respond to mechanical loading, and will provide a baseline of information from which we will, in the future, begin to explore how specific genetic alterations (e.g., via transgenic or knockout mice) affect mechanotransduction in bone. At the applied level, an outcome in which rest-inserted, low magnitude, non-invasive loading significantly augments and maintains murine cortical bone properties would place our group in position to begin to explore the efficacy of this concept in human subjects.

Herman, Gabor T | CUNY Graduate Sch And Univ Ctr | Image Processing In Biological 3d Electron Microscopy | HL070472 | 4 years | NHLBI

Abstract: DESCRIPTION (provided by applicant): Three-dimensional electron microscopy (3D EM) is a powerful technique for imaging complex biological macromolecules in order to further the understanding of their functions. It is achieving high goals and exceeding expectations unthinkable only a few years ago. However, there are still some problem areas where either not enough work has been invested or the work has not as yet been fruitful. A multidisciplinary approach is proposed to shed light on three of these areas by the application of image processing techniques: (i) Incorporation of realistic image formation models into new reconstruction algorithms which take into account image blurring models of the aberrations of the electron microscope and which are at the same time noise-resistant and flexible with respect to the different data collection geometries. (ii) Incorporation of knowledge regarding the specimen obtained by means other than EM, such as high resolution surface relief information and information regarding the chemical nature of the specimen. (iii) Improvement of the rendering and the analysis of the reconstructed volumes by the development of more accurate segmentation (of the specimen from its background) and visualization algorithms. These basic aims are to be complemented by a rigorous approach to validating claims of superiority of any of the newly developed methods over those used in current practice. The approach will include very realistic simulations of the electron microscopic imaging process on structures in the Protein Data Bank. Image processing methodology for obtaining more accurate structural information by 3D EM than what can be achieved by current techniques will contribute to our understanding of the detailed molecular mechanisms of some of the key cell functions and, consequently, impact on the field of drug discovery. The proposed work is relevant to cardiovascular and pulmonary disease and health and to blood research.

Hoh, Jan H | Johns Hopkins University | Nanofabrication of high performance AFM cantilevers | GM064020 | 3 years | NIGMS

Abstract: DESCRIPTION (provided by applicant): The long-term objective of this proposal is to develop high performance atomic force microscope (AFM) cantilevers for application to biomedical research. The AFM is emerging as a powerful tool for biomedical research, and has found applications that include imaging of DNA, measuring local surface electrostatics on membranes, mapping mechanical properties of cells and single molecule protein mechanics. It is also considered a central enabling technology in nanotechnology. One of the limiting elements in current AFMs is the cantilever. The performance of a cantilever is primarily characterized by a combination of spring constant and resonance frequency. We propose to develop new cantilevers with resonance frequencies in the range 1-100 MHz in solution, for cantilevers with a spring constant of 0.1 N/m. This resonance frequency is approximately one to three orders of magnitude better than the best cantilevers that are currently available. The new cantilevers will be constructed by two independent methods, focused ion beam milling of conventional silicon or silicon nitride cantilevers and electron beam deposition. Cantilevers with leg a thickness of 100 nm or less will and widths of 100 nm or less will be milled using an ion beam from the material at the end of a conventional cantilever, producing a compound cantilever with a small high performance cantilever at the free end of the larger one. Similar compound cantilevers will be constructed by electron beam deposition plastic like nanostructures in the shape of small cantilevers. Among other things, these new cantilevers will allow faster scanning, increase the temporal resolution of force measurement, improve measurement sensitivity by reducing cantilever noise, and improve sensitivity by reducing cantilever spring constant. To use these new cantilevers, we propose to construct an AFM head with appropriate optics to work with very small cantilevers and position sensor with data acquisition system with a detection bandwidth of at least 100 MHz. This detector will be used to characterize physical properties of the new cantilevers. In addition, we will test the performance of the new cantilevers in electrostatic mapping experiments where we expect improve sensitivity and a bilayer fusion experiment in which we expect to uncover new dynamics lipid rearrangement during fusion.

Humphrey, Jay D | Texas A&M University System | Mechanical Stress and the Heat-Treatment of Soft Tissue | HL068118 | 3 years | NHLBI

Abstract: DESCRIPTION (Provided by Applicant): The ever-increasing clinical use of heat to treat disease and injury has been driven primarily by advances in laser, micro-wave, radio-frequency and similar technologies, not a fundamental understanding of the biothermomechanics. Heat is used, e.g., in cardiology, dermatology, gynecology, oncology, ophthalmology, orthopedics, and urology. Recent uniaxial studies in our lab reveal that whereas increasing temperature hastens the thermal-damage process, increased loading during heating delays it. Fortunately, the potential complexity of this coupling is simplified by our discovery of a 1-0 time-temperature-load equivalency. The same outcome can be achieved via a multitude of different combinations of heating and mechanical loads if the duration of a non-dimensional (scaled) heating time is the same. This scaling is done by dividing the actual heating time by a temperature- and load-dependent characteristic time, which exhibits an Arrhenius behavior. This reveals that loading influences the process via the activation entropy, not energy. No prior clinical trial of a heating device or strategy has accounted for this coupling. Without such information, optimization will remain elusive. Note, therefore, that most tissues and organs are subjected to multiaxial stresses, thus there is a need to quantify the effects of multiaxial stresses on the thermal process as well as the effects of thermal damage on the multiaxial mechani-cal properties. No such data are available. Rather than focusing on a particular clinical protocol, the goal of this work is to assess the biaxial thermomechanics of collagenous membranes. We shall focus on collagen since it is the primary structural protein in the body, and thus it is present in most tissues that are heat-treated clinically and it is responsible for most of the post-heating structural integrity. Achieving our aims will have much broader impact, however. We recently showed that data from the literature on cell death and the denaturation of other proteins exhibit the same characteristic time-temperature equivalency that we discovered via tests on collagen -- we expect the current findings to similarly provide qualitative insight into the multiaxial thermomechanical behavior of many tissues. The possible multiaxial states of stress that can exist in vivo, or that can be induced by clinical interventions, is almost unlimited. We submit that the most prudent approach to developing a multiaxial theory that has broad predictive capabil-ity is to perform a broad series of isothermal biaxial-isotonic and isothermal biaxial-isometric tests. These data will be sufficient to formulate the requisite constitutive relations, which in turn will be evaluated further using data from combined isometric and isotonic test conditions and non-isothermal heatings. These consitutive relations will allow future evaluation of candidate protocols, from which the most promising can be chosen for animal testing I clinical trials. Without a firmer understanding of the effects of multiaxial stress on thermal-damage processes, we will continue to evaluate particular clinical devices and strategies by trial-and-error. There is a need for a firmer scientific understanding.

Jarrell, Kevin A | Boston University | Gene Engineering And Combinational Biology | AI048665 | 1 years | NIAID

Abstract: DESCRIPTION (Investigator's Abstract): The research program described in this application is focused on the development and implementation of new gene engineering methods. The methods that are being developed are particularly suited to the manipulation of modular genes, such as peptide synthetic genes. Our goal is to break the modular genes up into their component modules, and use those modules as building blocks for the assembly of novel new modular genes. To achieve this goal, we have developed two new approaches to gene engineering. Both approaches will be optimized and implemented during the funding period. The first approach involves the use of ribozymes(i.e., enzymes comprised of RNA) as tools for chimeric gene assembly. The second approach involves the use of RNA-overhang cloning (ROC) and NA-overhang cloning (DOC) to create chimeric genes. Finally, the ribozyme method will be combined with the ROC and OCmethods to create a new gene engineering system that takes full advantage of the strengths of the individual approaches. Efficient gene engineering methods are of fundamental importance for both basic and applied research on topics directly relevant to human health. For example, the gene engineering methods described here will be used to create combinatorial gene libraries that encode chimeric peptide synthetase genes. Naturally occurring peptide synthetases are known to synthesize important antibiotics, such as penicillin and vancomycin. Furthermore, the immunosuppressant cyclosporine is produced by a peptide synthetase. The chimeric peptide synthetase genes generated during the course of this project should encode hybrid enzymes that synthesize novel biologically active-molecules. A long-term goal of this project is to screen chimeric gene libraries to identify enzymes that synthesize novel compounds at could be developed as drugs.

Kilgore, Kevin L	Case Western Reserve University	A Novel Waveform for Electrical Nerve Conduction Block	NS040553	3 years	NINDS

Abstract: DESCRIPTION: (Verbatim from application) The goal of this research is to develop a reversible method for chronically blocking the conduction of action potentials in human peripheral nerves. Unwanted or uncoordinated generation of nerve impulses is a major factor in many disabling conditions, such as peripheral pain, spinal cord injury, stroke, cerebral palsy and multiple sclerosis. For example, unregulated nerve impulses produce spasticity in stroke, cause spasms in spinal cord injury, and generate neuroma pain in amputation. If these impulses can be intercepted along the peripheral nerves over which they travel, then the disabling condition can be reduced or eliminated. Although there are a few existing methods for surgically or pharmacologically blocking nerve impulses, none of these methods are broadly applicable or successful, are non-specific with sometimes serious side-effects, and, in many cases, are destructive to the nerve. Therefore, there is a widespread clinical need for a safe, reliable and reversible nerve block. The use of electrical stimulation, delivered through electrodes surrounding the nerve, has previously been shown to block nerve impulses in a reversible and predictable manner in acute situations. However, the present methods of electrical nerve block are likely to be damaging to the nerve during chronic usage. A novel stimulus waveform has now been developed that is likely to be safe for chronic human applications, while still producing an effective and reversible nerve conduction block. In this project, the effectiveness of this waveform to block action potential propagation in whole nerves in acute in-vivo experiments will be measured. Specifically, it will be demonstrated that this new waveform is capable of a complete block of both motor and sensory activity, including A-delta and C-fiber activity, and that this new waveform can also be used to selectively block activity in large diameter axons. The effect of nerve diameter and nerve fiber size on block effectiveness will also be evaluated. At the completion of this project, it will have demonstrated that an electrical nerve block can be achieved, and that it is effective in blocking conduction in both motor and sensory nerve fibers. In the future, chronic in vivo studies will be performed to test the long-term safety of this technique prior to human use. The initial intended human application will be to alleviate pain in individuals with neuromas secondary to limb amputation.

Kirz, Janos	State University New York Stony Brook	High Resolution 3D X-ray Diffraction Microscope	GM064846	4 years	NIGMS

Abstract: The three dimensional imaging of a small cell by an extended form of X-ray crystallography (or equivalently the development and use of a soft X-ray diffraction microscope for use in cell biology) is proposed. This instrument is designed to provide three-dimensional images of frozen hydrated cellular and sub-cellular structures at better than 20 nm resolution. The instrument does not use optical elements to form the image instead it records the diffraction pattern of the coherently illuminated object, and using techniques borrowed from crystallography, performs the reconstruction using an iterative algorithm. This way the resolution is not limited by the optics, and future developments should improve the resolution limit further. The diffraction pattern from a non-crystalline specimen is a continuous (speckle) pattern. Unlike the case with crystals this pattern contains sufficient information to overcome the phase problem of crystallography by sampling the diffraction pattern at a finer scale. Undulator radiation at the National Synchrotron Light Source is used to provide coherent illumination of the specimen. The diffraction pattern is recorded using a CCD detector. Special care is taken to shield the detector from all but the desired information. A single pattern yields a two-dimensional image. To obtain three-dimensional reconstruction the specimen is rotated and a set of diffraction patterns is collected. Frozen hydrated specimens are used to minimize the effects of radiation damage.

Kohn, David H | University Of Michigan At Ann Arbor | 3-D Biomimetic Scaffolds For Bone Tissue Engineering | DE013380 | 5 years | NIDCR

Abstract: DESCRIPTION (Adapted from the Investigator's Abstract): Reconstruction of skeletal defects represents a major clinical challenge with over 1 million surgical procedures performed each year. New strategies of regenerating bone are needed because of limitations with existing techniques. One new strategy is to create a composite graft in which autologous cells are seeded onto a porous, degradable scaffold. The scaffold supports the cells, structurally and biologically, allowing them to grow and secrete new extracellular matrix. Optimally tissue growth occurs concurrent with scaffold degradation. The degree of new bone formation is, however, material dependent and not predictable. We therefore seek to establish material chemistry parameters that could optimize bone cell function. In pursuit of this goal, we have developed: (1) in vitro culture methods in which human bone marrow stromal cells (BMSCs) are expanded; (2) polymer processing techniques to reproducibly fabricate highly porous 3D poly(lactic-co-glycolic) scaffolds, which have been successfully used to engineer a number of tissues including bone; (3) materials science design strategies which enable us to biomimetically modify both the internal microenvironment of a scaffold and the scaffold surface; and (4) a critical size cranial defect model in an immunocompromised mouse which has shown that the human BMSCs are capable of forming new bone in an animal model. The global hypothesis of the proposed research is that the extracellular microenvironment provided by the scaffold modulates the ability of human BMSCs to differentiate toward an osteoblast phenotype, and therefore controls biomineralization and structural integrity of regenerated bone. Results from our and other laboratories support this hypothesis, which is tested by synthesizing a series of model biomimetic materials. First, we synthesize environmentally responsive or "smart" scaffolds that buffer the microenvironment upon scaffold degradation. Second, we synthesize scaffolds with a surface that self-mineralizes into a biological apatite. Third, we use functionally-graded scaffolds in which mineralization is spatially controlled. The rationale for each of these 3 biomimetic strategies lies in the way nature has designed the skeleton. The skeletal system is able to perform its functions using a minimum amount of mass because biology has utilized design approaches, which include the ability to adapt to environmental cues (i.e. "smartness"), a hierarchical organization consisting of elegant mineral synthesis, and an organization that is optimized for physiological function by having gradients in composition and structure. In the proposed studies, we aim to exploit aspects of each of these 3 biomimetic strategies in an effort to create biomaterials that will modulate biological response in a controlled manner.

Kwon, Glen S | University Of Wisconsin Madison | Biospecific Polymer Enzyme Conjugates For Drug Delivery | CA086010 | 4 years | NCI

Abstract: Description: (Applicant's abstract) Once a target location has been identified for a drug, protein or gene, proper spatial and temporal control in the delivery of these molecules is a fundamental problem in biomedical engineering. In one promising approach for anticancer drugs called antibody-directed enzyme prodrug therapy (ADEPT), a monoclonal antibody (MAb)-enzyme conjugate selectively binds an antigen expressed on tumor cells, and the enzyme moiety releases drug from the subsequently injected prodrug at the target site. ADEPT is in clinical trials. However, drawbacks of MAb-enzyme conjugates limit ADEPT. They express low chemical and physical stability, short blood-half life, immunogenicity and low tumor to blood ratio. Attaching a common water-soluble polymer, methoxy-terminated poly(ethylene glycol) (PEG), onto MAb-enzyme conjugates enhances stability, prolongs blood circulation and reduces immunogenicity, but with no marked increase in tumor to blood ratio. Our research will focus on biospecific polymer-enzyme conjugates and their role in ADEPT. We attached a biotinylated PEG on a model enzyme, carboxypeptidase A (CPA). A biotin moiety at a chain end of PEG may mediate several useful functions for the first time for a PEG-enzyme conjugate. A biotin moiety may mediate the separation of PEG-CPA conjugate by affinity chromatography, fractionating in terms of number of attached biotinylated PEG on CPA using an immobilized monomeric avidin. A biotin moiety may tether an antibody in conjunction with biotin and streptavidin. Lastly, a biotin moiety may bind a clearing agent (e.g., streptavidin) in blood, an interaction that may mediate the clearance of biotinylated PEG-CPA conjugate by the liver and an increase in its tumor to blood ratio. The specific aims of the proposal: (1) To prepare a biotinylated PEG-CPA conjugate with controlled levels of biotinylated PEG at varied molecular weight by reductive amination and by affinity chromatography with immobilized monomeric avidin. (2) To study the catalytic activity and the stability of fractionated biotinylated PEG-CPA conjugates. (3) To study the immunogenicity and the plasma profile of fractionated biotinylated PEG-CPA conjugates in mice, focusing on their clearance by streptavidin. (4) To tether an IgG1 (174H.64) together with biotin and streptavidin on biotinylated PEG-CPA conjugate with optimized properties, purify the conjugate to obtain a 1:1 complex, study its stability, and assess target cell binding in vitro. (5) To study the plasma profile and the biodistribution of antibody-biotinylated PEG-CPA conjugates ("active" targeting) and biotinylated PEG-CPA conjugates ("passive" targeting) in tumor-bearing mice (KLN-205), assessing the effect of injected streptavidin.

Larson, Ronald G | University Of Michigan At Ann Arbor | A Microfabricated Device for Rapid Viral Genome Analysis | AI049541 | 3 years | NIAID

Abstract: DESCRIPTION (Applicant's abstract): We propose to develop a portable, self-contained, microfabricated device for extraction of genomic information from RNA or DNA viruses. Initially, we choose as a model system and as an important target the hemagglutinin HA1 of influenza A virus. Influenza is a prevalent human pathogen with an RNA genome. Mutations in the hemagglutinin (HA1) domains of influenza regularly produce new virulent forms that are responsible for 6 percent of annual mortalities in the U.S.A. Seasonal changes in influenza HA1 have a major impact on influenza epidemics and public health, and pose an on-going threat of world-wide pandemic. From analyses of influenza virus evolution, 18 of the most dangerous mutation sites have been identified. A present need is a reliable means to rapidly survey domestic and foreign populations for the emergence of new mutations. A self-contained, inexpensive, microfabricated device that can rapidly detect viral mutations using a small amount of sample would address this need. To expedite development of such a device we will perform research to achieve the following specific aims: Aim 1 - Determine the Influenza-A RNA purity requirements for Aims 2-4 by preparing samples of three levels: (a) cultured viral-infected cells, (b) purified whole viral particles, and (c) purified viral RNA. Aim 2 - On a microfabricated device, reverse transcribe and amplify the HA1 hemagglutinin domain of Influenza A using reverse-transcription PCR to produce double-stranded complementary DNA. Aim 3 - On a microfabricated device, perform fluorescent primer extension reactions on double-stranded DNA produced in Aim 2 to detect variations in bases in codons from the HA1 domain of hemagglutinin that have been involved in past viral mutations. Aim 4 - On a microfabricated device, separate primer-extended DNA products by gel electrophoresis and identify the locations of the base variations. Aim 5 - Integrate RNA separation, RT-PCR, primer extension reactions, electrophoretic separation, and (if necessary) RNA purification on a single microfabricated device. Aim 6 - Develop a silica gel RNA-adsorption column for purification of RNA on a microfabricated device.

Lewis, Nathan S	California Institute Of Technology	Biomedical Application Of An Electronic Nose	DC004712	4 years	NIDCD

Abstract: The focus of this proposal will be to exploit the vapor detection technology developed recently at Caltech that forms the basis for a low power, simple, manufacturable, "electronic nose". In this technology, an array of sensors responds to essentially all vapors, but produces a distinguishable response pattern for each separate type of analyte or mixture, much like the mammalian olfactory sense produces such diagnostic patterns and then transmits them to the brain for processing and analysis. Pattern recognition algorithms and/or neural network hardware are used on the output signals arising from the electronic nose to classify, identify, and where necessary quantify the vapor or odors of concern and to associate them with certain disease states associated with volatile biomarkers in the breath or other headspace samples. Due to the present high sensitivity of our electronic nose to biogenic amines (detection levels of 1-10 ppt in a few seconds in room air), which far exceeds that of humans for this class of compounds, we plan to initially explore the use of the sensor arrays to screen for bacterial vaginosis, which has been positively associated with the presence of "fishy" odors that arise from volatile biogenic amines in the headspace above vaginal swabs. In addition, we will advance the science and technology of the electronic nose sensors to obtain still improved sensitivity and time response for other biomarkers, so as to open up further medical application areas and to respond to potential confounding interferences identified by the initial small-scale clinical studies on the targeted, initial demonstration application of the technology.

Lindsay, Stuart M	Arizona State University	New SPM Methods to Study Chromatin Remodeling	CA085990	5 years	NCI

Abstract: DESCRIPTION(provided by applicant): It is becoming increasingly clear that promoter chromatin structure and the remodeling of that structure in association with gene activation are crucial facets of eukaryotic transcriptional regulation. The recent development of an in vitro MMTV-LTR system that can reconstitute the correct promoter chromatin structure and the correct remodeling of that structure in vitro presents an unprecedented opportunity to study these important facets of transcription regulation. In particular, it will now be possible to study promoter chromatin with and without bound receptor, and thus obtain information on this key first step of promoter recognition. We can then analyze the remodeled chromatin, to characterize the chromatin structure and transcription factor changes that have occurred as a result of remodeling. Because of its scale of imaging, the atomic force microscope (AFM) is well suited for studying the various structural aspects of this process that we want to analyze: the Linear Organization (nucleosome locations), the Higher-Order structure (conformations of fully hydrated chromatin and transcription factors), and Molecular Recognition Mapping (identifying specific molecules in the spreads based on antibody recognition). These approaches are a blend of established techniques and new AFM techniques that will be developed for this application but will undoubtedly prove useful for other biophysical and biological applications. The new techniques will be developed using known and defined model chromatin templates (e.g., 208-12 and known modifications thereof) and well characterized transcription factors. These techniques will then be applied to study the MMTV-LTR promoter chromatin structure and the remodeling of that structure and the results of the control, model systems used to provide a baseline to help interpret the MMTV-LTR results. The proposal brings together a physicist (Stuart Lindsay, Arizona State University, scanning probe microscopy, instrument development) and biochemists (Gordon Hager, NCI, chromatin remodeling, Dennis Lohr, ASU, transcriptional regulation/chromatin structure and Rodney Harrington, ASU, DNA-protein interactions) to develop techniques, test them on control samples and then apply them to remodeling of promoter chromatin. The collaboration will include biophysicists Hansgeorg Schindler and Peter Hinterdorfer (University of Linz, Austria) pioneers in developing nm-scale molecular recognition techniques that use an antibody attached to an AFM probe. Instrument development will be supported by Molecular Imaging Corporation.

Macovski, Albert	Stanford University	Development of A Prepolarized MRI Extremity Scanner	CA092409	1 years	NCI

Abstract: The objective of this research project is to demonstrate the technical feasibility of an ultra-low-cost form of magnetic resonance imaging (MRI) called Prepolarized MRI for human extremity imaging. Conventional MRI systems cost between 1 million dollars and 3 million dollars. We propose a novel Prepolarized MRI system that uses two inexpensive electromagnets rather than one expensive superconducting magnet. We aim to demonstrate that a complete dedicated extremity Prepolarized MRI scanner imaging can be constructed for less than 50,000 dollars while maintaining excellent image quality. Over the last five years, we have made excellent progress towards these goals, including our first in vivo human wrist images recorded earlier this month. The total capital cost of our 0.43 T Prepolarized MRI wrist imaging system was less than 35,000 dollars, excluding the MRI data acquisition console. Our principal aim is to expand the size of the imaging system to 31 cm to accommodate knees, feet, and elbows. Once basic imaging is achieved on the 31-cm-bore system, we plan several hardware and software improvements to increase the SNR and contrast flexibility. We also plan a monthly study of normal human knee cartilage conspicuity. Two Stanford Radiologists will compare the normal images with gold-standard images obtained from a 0.5 T GE extremity scanner, and gauge whether the previous month's engineering changes actually improved image conspicuity. We hope at the end of this four year grant to have constructed a 0.5 T Prepolarized MRI system with SNR and contrast comparable to a conventional 0.5 T MRI scanner for less than 50,000 dollars in capital costs. If successful, our research could ultimately lead to significantly greater availability of high-quality Prepolarized MRI scanners, which could be sold for less than the cost of an ultrasound scanner.

Mahvi, David M	University Of Wisconsin Madison	Hepatic Rf Ablation: Development Of Effective Devices	DK058839	5 years	NIDDK

Abstract: Radiofrequency (RF) ablation can be delivered percutaneously to hepatic tumors in a minimally invasive manner. The major limitation of RF ablation has been a high post- treatment local tumor recurrence rate. The most troublesome reason for the high local recurrence rate associated with RF, the inability of RF to destroy all tumor cells within a zone targeted for ablation, is the focus of this proposal. Preliminary finite element modeling (FEM) of a commonly used RF device has suggested that tumor cells within an RF lesion, but adjacent to vessels, were not heated to lethal temperatures. We have developed an implantable in vivo animal model of solitary liver metastasis. In this model RF ablation sometimes yielded incomplete tumor cell death within the RF lesion. We therefore hypothesize that RF, as currently delivered, does not kill all tumor cells within a lesion. We propose to develop FEMs of RF ablation that predict current and temperature distribution in hepatic tumors. These models will incorporate RF catheter specifications, thermal convection (effect of blood flow), tissue resistivities, and tissue thermal conductivities. Commercially available devices will be modeled and the results confirmed in both a solitary metastasis model, and a model of tumor adjacent to large vessels. We further hypothesize that FEM can be utilized to design better RF devices and procedures, and thus optimize cell death. RF delivered by new electrode designs, at different frequencies, and via multiple devices will be modeled. Biomedical engineers will focus on the FEM and construction of different RF probe systems utilizing electrical resistivity data and thermal tissue properties. When a promising design is found, in vivo experiments to validate the mathematical model will be performed. The team we have formed is uniquely poised to address the clinical problem of ineffective RF ablation. Using these data, new RF units can be designed to overcome the limitations of currently available RF ablation systems.

Makrigiorgos, Gm M | Dana-Farber Cancer Institute | Microsphere Array for Lung Cancer Mutation Scanning | CA090422 | 2 years | NCI

Abstract: DESCRIPTION (Applicant's Abstract): Genome-wide screening for inherited polymorphisms (SNPs) and acquired mutations leading to cancer or to other genetic diseases is instrumental for understanding the causes of the disease and the likely response to treatment. However, the ability to detect 'en masse' such genetic alterations is currently hampered by the lack of technologies able to screen for unknown mutations in large gene pools simultaneously. The aim of this proposal is to engineer a novel microsphere-based DNA array which, in conjunction with a new mutation-scanning technology (ALBUMS), allows highly parallel screening of unknown mutations/SNPs in thousands of genes simultaneously. A pilot application in lung cancer is proposed. cDNAs derived from cancerous and normal cells are annealed and hybridized to generate mismatches at the positions of mutations/SNPs. ALBUMS attaches molecular probes covalently at unique chemical groups (aldehydes) generated at the mismatches by highly specific mismatch-repair glycosylases. Mutation -containing DNA is then isolated from normal DNA, PCR-amplified and applied on novel microsphere-based DNA arrays for single-step identification of the mutated gene regions. The combination of ALBUMS with microsphere-based arrays leads to a rapid pre-screening method for the entire cDNA, which can indicate multiple mutated gene regions commonly present in a patient population, which are then sequenced. This new approach leads to a dramatic reduction in the effort required to define mutations crucial to cancer development. Aims 1 and 2 will design, engineer and optimize an array of 100 sets of oligonucleotide-coated, optically encoded microspheres appropriate for the simultaneous identification of 100 ALBUMS-isolated, mutated DNA fragments. Multiplexing and extracting information from all 100 sets of microspheres simultaneously, by using a flow cytometer, will be optimized for minimal false positives/negatives. Aim 3 will expand the array by engineering microspheres appropriate for the simultaneous mutation/SNP analysis of 1000-2000 cancer-related genes. Finally Aim 4 will apply this approach to the high-throughput analysis of lung adenocarcinoma samples from 50 patients. Mutation/SNP-containing fragments that show potentially significant trends (e.g. mutations commonly present in high percentage of patients) will then be sequenced to define the exact position and nature of the mutation. If the engineering and application of the present array proves successful, future work will automate the production of microsphere-based DNA arrays in order to simultaneously screen for mutations/SNPs in the entire human genome.

Margulies, Susan S | University Of Pennsylvania | Biomechanics of Pediatric Head Injury | NS039679 | 5 years | NINDS

Abstract: Head injury is a leading cause of death and acquired disability in childhood. However, the biomechanics of pediatric head injury are poorly understood, primarily due to, the paucity of age-specific data regarding mechanical properties of immature tissue and its response to specific loads. The interdisciplinary proposed research plan is designed to answer the following question: What mechanisms cause what injuries in children of what age? The long-term objectives of the proposed research plan are to determine mechanical properties of the skull and brain, the loads they can withstand safely, and unique mechanisms for primary brain injury in infants (less than 3 months) and young children (1-3 years). In so doing, the long term impact of proposed research plan will be to open pathways for enhanced traumatic head injury prevention, detection, and treatment strategies specific to infants and toddlers. Both contact and non-contact mechanisms of brain injury will be investigated. The research plan uses an integrated bioengineering approach consisting of animal experiments, human and animal tissue tests, clinical studies, and anthropomorphic surrogates, all complemented by mathematical models to: A) measure pediatric tissue injury thresholds for acute neural, vascular, and blood-brain barrier damage B) measure pediatric skull and brain tissue mechanical properties C) create computational models for infant and toddler head injury using (A) and (B) D) qualitatively validate the computational model predictions with witnessed accidental head injuries in children E) measure loads experienced anthropomorphic surrogates during falls, shakes, and inflicted impacts F) determine the relative roles of impact forces and inertial loads in the etiology of primary brain injuries G) compare the computational simulations with acute clinical data to infer potential mechanisms of injury in non-accidental head injury. The overall hypotheses of the proposed research program are that 1) thresholds for skull fracture and tissue injury and mechanical properties of the brain and skull vary with age, such that both contribute to differences in primary head injuries between infants and toddlers, and 2) the increased compliance of the infant skull results in greater brain tissue injury from impact trauma; and 3) a valid computational model can be created to predict specific primary injuries resulting from a given reported mechanism.

Marro, Kenneth I | University Of Washington | NMR Measurement of Perfusion in Skeletal Muscle & Brain | HL064946 | 4 years | NHLBI

Abstract: DESCRIPTION (Provided by Applicant): Capillary-level perfusion is a key physiological parameter and its measurement is vital to the complete understanding of organ function. The usefulness of perfusion measurements is readily demonstrated by the wide range of methods currently used in research labs and medical practice. Almost all of these methods rely on the use of exogenous contrast agents, which suffer from a number of disadvantages that complicate interpretation and limit utility. We have developed a unique and completely non-invasive NMR perfusion measurement technique, which we call FAWSETS (Flow-driven Arterial Water Stimulation with Elimination of Tissue Signal) FAWSETS is sensitive enough to provide the temporal and spatial resolution required for clinical applications and specific to perfusion so that quantification is feasible with a simple model. The goal of this project is to further develop and optimize FAWSETS and demonstrate its value as a clinical as well as research tool. Specific aim #1 will focus on enhancement of perfusion sensitivity. This will require construction of custom RF/gradient coils and extensive testing in flow phantoms. In specific aim #2 we will conduct measurements in skeletal muscle that will define the specificity of the measurements in humans. Skeletal muscle is unique among organs in that prolonged ischemia can be safely and easily induced. This permits simple but vital tests for specificity that cannot be conducted in any other organ. Specific aim #3 will focus on sensitivity to perfusion. Our brain imaging experiments will demonstrate sufficient sensitivity for an important clinical application: the ability to monitor cerebrovascular reserve during the acetazolamide challenge. This project consists of a carefully considered plan to demonstrate the value of our method as a tool with potential for broad application. We are confident that our methods will later be adapted to test physiological hypotheses in a variety of organs where unambiguous information on parenchymal perfusion is needed. Our specific aims focus on two such organs. We have chosen to develop the measurements in skeletal muscle and brain because of their particular circulatory properties and because both organs are studied routinely and extensively in this research group. Our intention is to expand this work into significant clinical and basic science programs in future grants.

Matthew, Howard W | Wayne State University | Non-Differentiative Hematopoietic Stem Cell Expansion | DK058711 | 5 years | NIDDK

Abstract: DESCRIPTION (provided by applicant): The routine use of hematopoietic stem cell (HSC) transplantation as an effective treatment of hematologic and malignant diseases is hampered by the limited availability of HSC-enriched cell populations In addition, the success rate of HSC transplant therapies could be significantly increased if large quantities of transplant material with a high HSC content could be reliably generated. An increased HSC supply would also greatly stimulate research activities based on gene therapy of the hematopoietic system. The long term goal of this project is to address these limitations by developing a well defined culture system capable of specifically expanding an HSC-enriched progenitor population while simultaneously maintaining or increasing the HSC percentage. In most hematopoietic culture and expansion schemes, expansion and lineage differentiation proceed in parallel and HSC expansion per se is limited or non-existent. Recent studies suggest that in addition to manipulation of the cytokine milieu, it may be possible to direct the differentiation vs. proliferation activities of hematopoietic populations by controlling the nature and levels of glycosaminoglycan (GAG) substances to which the cells are exposed. Our previous work showed that this approach can be used to generate very high yield expansions of umbilical cord blood progenitors while simultaneously maintaining and enriching the culture with CD34+ cells. In the proposed studies, we will: (1) fully characterize and refine our culture system; (2) characterize the expanded cell populations with regard to HSC attributes; (3) develop and validate mechanistic mathematical models of GAG mediated hematopoietic control; and (4) use these results to design and optimize a perfusion bioreactor system based upon the polysaccharide surfaces. The bioreactor operating parameters will be optimized with the goal of producing, high yield expansion of cord blood progenitor populations. These studies will provide both fundamental knowledge about the poorly understood roles of various matrix polysaccharides in hematopoiesis, as well as the technology base for ultimately attaining specific non-differentiative expansion of pluripotent HSCs.

Maudsley, Andrew A | Northern California Institute Res & Educ | Proton Mr Spectroscopic Imaging Of Epilepsy | NS041946 | 1 years | NINDS

Abstract: Proton MR Spectroscopic Imaging (MRSI) enables non- invasive measurement of tissue metabolite distributions and offers considerable potential as a diagnostic imaging technique for localization of epilepsy, a devastating condition that affects thousands of children and adults. The proposed technique development is aimed at improving the effectiveness of these techniques for presurgical evaluation of epilepsy. The measurement of metabolite distributions in human brain is possible with only modest spatial resolution, for which conventional Fourier reconstruction methods result in errors associated with the truncated sampling. To improve the quality of the metabolite images, new reconstruction methods will be developed that do not suffer from these limitations and which enable improved spatial resolution for reconstruction of stronger metabolite signals. This will be achieved by using a Bayesian framework to incorporate known spatial and spectral information into an optimization reconstruction procedure. Although computationally intensive, these new methods can now be practically applied with the availability of low-cost multiprocessor computers. A second aim of this proposal is to develop methods for measurement of brain pH distributions using proton MR observation, which will provide additional diagnostic information as well as improving understanding of metabolic changes associated with epilepsy. This will be achieved by using a signal enhancement technique based on the administration of histidine and development of specialized parametric spectral analysis procedures. This measurement will offer increased sensitivity over previously used phosphorus measurements, as well as providing the capability for pH measurement on standard clinical MRI instrumentation. The developed MRSI techniques will be evaluated for detection of focal metabolic abnormalities associated with epilepsy. The improved metabolite image reconstruction and regional pH measurement techniques also have potential clinical applications in other areas, such as cancer, stroke, and brain trauma.

Merfeld, Daniel M | Massachusetts Eye And Ear Infirmary | Adaptation To Controlled Vestibular Stimulation | DC003066 | 5 years | NIDCD

Abstract: Over 90 million Americans (greater than 40 percent) will seek medical attention for dizziness or some other balance disorder sometime in their life. A NIH working committee recently reported that at least 2 million Americans experience chronic impairment due to dizziness or other balance disorders, causing medical expenses in excess of 1 billion dollars per year. Many of these chronically impaired patients could benefit from vestibular rehabilitation, and some of these patients could benefit from a vestibular prosthesis (similar to the cochlear implant for profound sensorineural hearing loss). This proposal directly addresses both of these health care needs. Specifically, the proposed studies develop and test a prototype neural prosthesis. Furthermore, to enhance our understanding of vestibular adaptation, the proposed studies use this prototype device to investigate adaptation to changes in peripheral vestibular stimulation. This study will be the first to comprehensively investigate adaptation to changes in chronic, peripheral stimulation of the vestibular system. A better understanding of vestibular adaptation will lead to improved vestibular rehabilitation. These general scientific goals will be achieved by investigating the following specific aims: 1. Study the importance of bilateral versus unilateral cues. 2. Study how the nervous system adapts to changes in peripheral stimulation of the branch of the vestibular (VIIIth) nerve that innervates the lateral semicircular canal. 3. Study how the nervous system combines sensory information from the otolith organs and semicircular canals when the rotational cues are provided via electrical stimulation. 4. Study how the nervous system adapts to yaw rotational cues delivered to a nerve branch innervating one of the vertical canals that does not normally include yaw rotational information. 5. Study how the nervous system adapts to constant-rate electrical stimulation while stationary, with and without visual cues. All of these proposed specific aims will be investigated by measuring changes in the vestibulo-ocular responses induced by changes in chronic, patterned, electrical stimulation of the peripheral vestibular system.

Mourant, Judith R | University Of Calif-Los Alamos Nat Lab | Raman Spectroscopy For Cancer Diagnosis And Monitoring | CA089255 | 5 years | NCI

Abstract: DESCRIPTION (Verbatim from Applicant's Abstract): Our general objective is to enable the application of vibrational spectroscopy to cancer diagnosis and treatment monitoring. The detection of cancer at its earliest stages is crucial, for it greatly improves the likelihood of successful treatment. Traditional methods of diagnosis have relied on physical removal of a portion of tissue and microscopic assessment of morphology. The need for tissue removal reduces the area of tissue that can be sampled. A noninvasive technique would eliminate this problem. Furthermore, a noninvasive technique has the potential to allow treatment to begin during the same endoscopic procedure used for diagnosis and reduce other complications associated with tissue removal such as tissue handling and increased risk of infection to the patient. We will focus on developing Raman spectroscopy for detection of precancerous conditions in patients with Barrett's esophagus. Barrett's esophagus is a pathology in which the squamous epithelial lining is replaced by a specialized metaplastic epithelium and the likelihood of adenocarcinoma is increased. Because the microscopic changes of dysplasia are difficult to observe, the entire area of metaplastic epithelium should be sampled. Therefore, Barrett's esophagus is well-suited for a noninvasive diagnostic technique. The methods and techniques developed in this proposal may also find application in other tissues such as the cervix. The second goal of our work is to develop Raman spectroscopy as a method for assessing the effects of treatment. Current methods for monitoring the response of an individual tumor to therapy are unreliable and often difficult to implement during the course of therapy. Development of noninvasive or minimally-invasive optical methods to reliably identify regions of apoptosis and necrosis would provide a simple method for assaying tumor response in each individual cancer patient. Consequently, treatments could be customized.

Ong, Joo L | University Of Texas Hlth Sci Ctr San Ant | Early Bone Formation At Bone Biomaterial Interface | AR046581 | 5 years | NIAMS

Abstract: DESCRIPTION (Verbatim from the Applicant): The goal of this research is to gain a better understanding of the biological basis for successful orthopaedic and dental implant therapy by elucidating the early phenomena that govern osseointegration. In this proposal, the effect of sputtered hydroxyapatite (HA) crystallinity on early bone cell activity in vitro and in vivo will be investigated under highly controlled and defined conditions. Our overall hypothesis is that, under conditions where other variables are controlled, the degree of crystallinity of the HA surface directly affects early bone cell activity in vitro and the rate of development of osseointegration in vivo. The objective of this study is to correlate the effect of characterized HA crystallinity to dissolution and protein adsorption, bone cell response in vitro and early cell activities in vivo. In this proposal, Aim 1 will be to determine the relationship between crystalline content of well-characterized HA surfaces and 1) the adsorption of specific extracellular matrix proteins, fibronectin and osteopontin, and 2) the rate of dissolution of the surface. The HA and Ti coatings will be produced using sputter coating. The rationale for using the sputtering technology is due to the high coating-metal adhesion strength compared to plasma spraying. Protein adsorption and dissolution of the coatings will be measured over time. Aim 2 will determine the extent to which the crystalline content of HA surfaces effects osteoblast proliferation, differentiation, and metabolism in vitro. It is hypothesized in this aim that because osteoblast proliferation and differentiation may be affected by either the adsorption of specific extracellular matrix proteins, fibronectin and osteopontin, or the rate of dissolution of the surface, or both; metabolic activity leading to mineral formation will vary with the crystalline content of the HA surface. Implicit in this hypothesis is there exists an optimal crystalline content of an HA surface for the promotion of bone formation activity. Aim 3 will evaluate the extent to which the crystalline content of HA surfaces affects osseointegration in vivo. Early bone activity will be evaluated using histology, mechanical strength and immunohistochemistry in this aim. Data generated from this study will provide information on the early maturation of bone cells in the presence of implant biomaterials and will provide a correlation between biomaterial properties and bone cell responses in vitro and in vivo. Additionally, information generated will contribute to the development of an ideal implant surface, thereby reducing long-term implant failures.

Palanker, Daniel	Stanford University	Electrical Alternative Lasers Intraocular Microsurgery	EY012888	3 years	NEI

Abstract: We propose to develop and evaluate a precise yet low cost electric operative cutting instrument for ophthalmic surgery based on pulsed plasma-mediated dissection of soft tissue in liquid medium. One of the first applications of this device will be in vitreoretinal surgery, namely, for tractionless removal of vitreoretinal membranes. The common techniques for treatment of vitreoretinal membranes are mechanical segmentation, peeling or delamination where a significant degree of traction is often applied to the underlying retinal tissue, and this can induce damage to the internal layers, iatrogenic tears and bleeding. Several attempts to develop laser- based instrumentation for vitreoretinal surgery have been undertaken, but all these systems have failed so far to achieve widespread acceptance due to either extensive collateral tissue damage, or high cost and low efficiency of these systems. One of the most powerful mechanisms of laser-tissue interaction in liquid medium is dielectric breakdown-based plasma generation. This approach, based on application of tightly focused short pulse lasers, has not been accepted clinically in vitreoretinal surgery due to difficulties with tight focusing of the laser beam near the retina in real operational conditions. We propose to use a similar interaction mechanism but without lasers. A sub-microsecond high voltage discharge applied via an intraocular microelectrode will generate plasma in liquid medium and can allow for precise cutting of soft tissue. The energy deposition is confined to the area determined by the size of the electrode - on the order of a few micrometers - thus allowing for very low threshold energy and very fine control of the penetration depth. This system combining high precision, reliability and versatility with low cost will allow for widespread acceptance in operating practice. Applicability of this approach to vitreoretinal surgery and other intraocular procedures, such as capsulotomy and cataract surgery will be tested in-vitro and on animal models including histological analysis, scanning electron microscopy and physiological tests.

Patwardhan, Abhijit R	University Of Kentucky	Cardio-Respiratory Interaction: Contribution in Syncope	HL065735	3 years	NHLBI

Abstract: Respiratory and cardiovascular regulatory systems share goals of homeostasis, however, dynamically inappropriate adjustments in one may precipitate undesirable consequences in other. Our general hypothesis is that dynamic cardio-respiratory interaction contributes importantly in genesis of syncope. Changes in respiratory patterns preceding syncope have been anecdotally reported for several years. Evidence from recent studies support these observations. We hypothesize the following chain of events: transient disturbances in arterial partial pressure of carbon dioxide (PCO2), precipitated by oscillations in perfusion, trigger ventilatory adjustments. These oscillations increase in amplitude during orthostasis. The ventilatory adjustments, in those with exaggerated chemical sensitivity to carbon dioxide, leads to increased ventilatory variability. The resulting periods of hyperpnea and hypocapnia, through changes, either or collectively, in cerebral blood flow, chemoreflex mediated sympathetic withdrawal, or altered intrathoracic mechanics, trigger hemodynamic instability, collapse and syncope. We will, verify whether exaggerated sensitivity to changes in chemical stimuli produces altered ventilation preceding syncope, quantify the role of two consequences of altered ventilation, chemical drive and intrathoracic mechanics, independently and together, in genesis of syncope. We will use head up tilt in humans, to 1) verify whether the dynamic ventilatory response to carbon dioxide perturbations in subjects who develop syncopal symptoms is exaggerated than those that do not, 2) quantify the effects of decrease in arterial partial pressure of carbon dioxide (PCO2), in the absence of changes in respiration, on cerebral vasoconstriction and syncope, and 3) quantify the effects of buffered changes in PCO2, on altered breathing, hyperpnea, cerebral vasoconstriction and syncope. Our study will provide results to answer these important questions; what causes the often observed increased ventilatory variability before syncope?, what are the effects on syncope of decreases in PCO2, while ventilation remains unchanged? what are the effects on syncope of changes in ventilation, while PCO2 remains unchanged? Collectively, these results will create knowledge to further understand the mechanisms of the debilitating and sometimes dangerous condition of syncope that affects upwards of 10 to 15 percent of young and adult population.

Peterson, Ellengene H | Ohio University Athens | Biomechanics of Vertebrate Hair Cells | DC005063 | 5 years | NIDCD

Abstract: Hair dells are the receptors that vertebrates us to detect sound, head movement, vibrations, and gravity. Each of these sensations begins with a mechanical stimulus. Hair cells respond to this stimulus via a complex cellular process that shapes the primary afferent signal to the central nervous system. The first step in this process and the one on which all others depend is deflect of the hair cell's ciliary bundle. Unfortunately, the mechanical and cellular mechanisms that govern this first critical step are poorly understood. The long term goal of the proposed research is to understand these fundamental mechanisms of mechanotransduction by hair cells and to develop a realistic computational model of this process. It is a collaborative bioengineering effort that uses state-of-the-art imaging and computational technology. The proposed research has three bioengineering effort that uses state-of-the-art imaging and computational technology. The proposed research has three Specific Aims. (1) We will use light and electron microscopic techniques to characterize quantitatively the structure of hair cells, emphasizing those features of their ciliary bundles that are likely to affect the hair ells' mechanical performance, and we will use brightfield and confocal microscopy to visualize the coupling between hair bundles and the overlying otolithic membranes in living utricles. (2) We will incorporate these data into a structurally accurate finite element model of the ciliary bundle that will quantify the contribution of different structural elements (e.g., number, height, and interconnections of stereocilia) and the in vivo stimulus to the static stiffness and response dynamics of morphologically distinct varieties of hair cells. Then we will test and refine our model predictions by experimental tests on living bundles. (3) We will use our computational model to predict current-displacement relations in bundles of different types. Then we will use whole-cell patch clamp recording from living hair cells to further test and refine our model predictions. These studies will provide, important information about mechanisms of mechanotransduction and the functional significance of ciliary bundle structure. The resulting computational model will be a powerful resource in future attempts to understand the mechanical performance of any vertebrate hair cells.

Petroll, Walter M | University Of Texas Sw Med Ctr/Dallas | Assessment Of Corneal Fibroblast Biomechanical Behavior | EY013322 | 3 years | NEI

Abstract: DESCRIPTION (Adapted from applicant's abstract): Cell-matrix biomechanical interactions play a critical role in both physiological and pathological processes such as embryonic tissue morphogenesis and wound repair. Despite general agreement that fibroblasts exert mechanical forces on the extracellular matrix (ECM) to promote organization of the collagen architecture, the underlying mechanisms of force transduction are not clearly understood. Based upon previous studies of in vivo corneal wound healing, we hypothesize that (1) mechanical forces are generated by a muscle-like contractile mechanism; and, (2) an isotropy in the ECM leads to a progressive alignment of this contractile machinery parallel to the axes of greatest mechanical resistance. We have recently developed a novel biophysical system that allows measurement of the forces generated by isolated corneal fibroblasts on a fibrillar collagen matrix and the direct correlation of ECM force vectors with specific cellular movements. Using this system, we have found that generation of force on the ECM correlates temporally with cellular contraction. While these pilot data are consistent with our original hypothesis, they do not yet definitively establish the role of contractile shortening in force generation, or the effect of anisotropy on cell alignment and tension generation. Thus, the overall goal of this Bioengineering Research Application is to develop a new, unique experimental system for directly and quantitatively correlating changes in protein organization with cellular force generation on fibrillar collagen matrix and to determine the effects of tissue anisotropy on the contractile response, using our existing biophysical system as a foundation. Our Specific Aims are to: (1) incorporate live-cell fluorescent imaging into our experimental model to allow simultaneous measurement of cell-induced matrix distortion and changes in the organization of contractile proteins; (2) use digital image analysis and finite element modeling to assess quantitatively the relationships between changes in contractile protein organization and cellular force generation; and (3) determine the effect of anisotropy in the ECM on the pattern of cellular force generation and contractile protein organization by inserting microneedles into the ECM in order to modulate matrix stiffness. This research should provide unique insights into the mechanical interactions between cells and ECM, and should serve as a critical foundation for future quantitative cell mechanics studies in this and other laboratories.

Roorda, Austin J | University Of Houston-University Park | Adaptive Optics Scanning Laser Ophthalmoscope | EY013299 | 3 years | NEI

Abstract: DESCRIPTION: In healthy normal subjects, the primary resolution limiting factors to retinal fundus imaging are the aberrations caused by the eyes' own optics. The applicant was among a team of investigators at the University of Rochester that first demonstrated that these aberrations could be measured with a Shack Hartman wavefront sensor and cancelled using a deformable mirror in a process called adaptive optics (AO). The applicant has now obtained funding from the NSF, University of Houston and Pharmacia and Upjohn to develop a similar device. This device would be incorporated into a scanning laser ophthalmoscope (SLO) rather than the more conventional fundus camera design used in Rochester. This SLO design has the potential to greatly decrease the effects of scattered light and further increase the resolution of fundus imaging.

Sacks, Michael S | University Of Pittsburgh At Pittsburgh | Enhanced Durability Of Bioprosthetic Heart Valves | HL063026 | 4 years | NHLBI

Abstract: DESCRIPTION (Verbatim from Applicant's Abstract): Porcine bioprosthetic heart valves (PBHV) continue to fail from calcification and mechanical damage. We have demonstrated for PBHV that cyclic fatigue induces loss of radial compliance, tensile strength, and flexural rigidity. Clinically, about 85 percent of all PBHV fail with tearing, and some fail with little or no calcification. These and other studies demonstrate that while not a strict prerequisite for calcification, maintaining tissue structural integrity is a prime factor in inhibiting PBHV calcification and extending durability. Our long-term goal is the development of rigorous engineering principals for improving PBHV, based on a thorough understanding of tissue-and organ-level biomechanics. During the cardiac cycle cusps undergo large flexural displacements, subjecting the layers to alternating tensile and compressive stresses. Cuspal flexural rigidity, and hence the stresses during flexure, are substantially increased by chemical treatment. Since PBHV are fibrous composite materials, it is likely that they are very susceptible to compressive stress induced damage. We hypothesize that a major mechanism of PBHV failure is structural damage independent of calcification, resulting from high compressive stresses present in the chemically treated tissue extracellular matrix (ECM) during cuspal flexure. An in-depth understanding of the fatigue life behavior of the chemically treated porcine aortic valve cusp independent of PBHV design is a critical first step towards the development of novel chemical treatments that seek to mitigate the effects of structural damage. This will ultimately aid in the rational development, as opposed to the current ad-hoc approach, of novel chemically modified collagenous biomaterials for more durable PBHV. We will test our hypothesis with the following specific aims: 1. Determine how chemical treatment alters cuspal layer micromechanics. 2. Quantify PBHV cuspal deformation during the cardiac cycle. 3. Determine how long-term cyclic fatigue alters PBHV later structure and mechanical properties.

Setton, Lori A | Duke University | Mechanical Stimulation Of IVD Cells | AR047442 | 5 years | NIAMS

Abstract: DESCRIPTION: (verbatim) Cells of the intervertebral disc (IVD) exhibit little capacity for functional matrix repair in situ, which may contribute to the progressive nature of disc degeneration. IVD cells respond to mechanical stimuli with altered biosynthesis in manner that depends on zone of cell origin (anulus fibrosus, transition zone or nucleus pulposus), although the mechanisms governing these responses are poorly understood. The central hypothesis of this proposal is that zonal variations in the local mechanical environment of IVD cells are dominant in regulating zonal variations in disc cell metabolism. Our preliminary analytical and finite element analyses suggest that the micromechanical environment of disc cells depends on four parameters: (1) the anisotropic, nonlinear and multiphasic properties of the extracellular matrix; (2) applied loading conditions; (3) cell mechanical properties; and (4) three-dimensional cell geometry. In this study, we propose a set of experiments to quantify zonal variations in these four parameters and to incorporate them in a computational model of IVD cell micromechanics. Independent tests will be performed to quantify material properties of the cell and extracellular matrix using materials testing and micropipette aspiration techniques. Three-dimensional cell geometry and the local boundary conditions in the IVD under compression will be obtained using confocal laser scanning microscopy. A finite element model of the micromechanical environment of IVD cells will be developed, which incorporates nonlinear, anisotropic and biphasic material behaviors and the measured material and geometric data. Finite element model predictions of the local mechanical environment of IVD cells under compression in the intact IVD (in situ), and isolated cells cultured in a three-dimensional alginate matrix (ex situ), will be obtained to precisely determine important local mechanical stimuli such as stress, strain, fluid pressure and fluid flow. To construct new and precise relationships between these stimuli and IVD cell metabolism, corresponding experiments to measure zonal variations in gene expression and biosynthesis of collagens and agrecan will be performed on intact IVD and cell-alginate constructs under compression. Comparison of data for in situ and ex situ experiments is expected to reveal the relative contributions of cell morphology, matrix properties and loading conditions to the micromechanical environment and metabolic response of IVD cells. Furthermore, the experimental and computational data to be obtained in this project will define new relationships between precisely determined mechanical stimuli and IVD cell metabolism that are prerequisite to understanding the mechanisms that govern cellular response to mechanical stimuli in vivo.

Tender, Leonard M | U.S. Naval Research Laboratory | Biosensor For Investigating A Developing Immune Response | AI047427 | 2 years | NIAID

Abstract: DESCRIPTION: Helper T (Th) cells are critical regulators of adaptive immunity. The cytokines produced by Th cells are powerful bioactive agents that control the outcome of an immnune response to most foreign protein antigens. Cytokine production occurs in complex waves that serve to regulate antigen-specific clonal expansion and subsequent differentiation of effector and memory lymphocytes in vivo. It is our hypothesis that development of sensitive miniaturized sensors able to detect and quantify multiple molecular species in real-time would significantly impact our fundamental understanding of immune regulation. The proposed study will focus on antigen-specific Th cell-dependent B cell development. A view of cytokine-regulated antibody production will be developed that is quantitative and provides a kinetic dimension to the production of multiple cytokines (IL2, IL-4, IL-5, IL-6, IL-lO, IFN-y and TNF-a) and subsequent secretion of multiple immunoglobulin (Ig) isotypes (IgM, IgGi, IgG2a, IgG2b, IgG3, IgE and IgA). The main objective is development of sensitive biosensors able to detect minute quantities of multiple cytokines over time. The first specific aim is proof of concept of an innovative application of capacitance-based biosensing in which microfabricated field effect transistors are modified with specific receptors. This proposed device amplifies the change in gate double-layer capacitance that occurs upon ligand binding - resulting in sensitivity at least three orders of magnitude greater than existing assays while generating a signal that is independent of the sensor area (allowing miniaturization and arrays). The second specific aim is evaluation of sensitivity, specificity and dynamic range of the proposed sensor for individual cytokine and Ig components described above using purified protein sources and timed addition in vitro. The production of these cellular products from defined populations of Th cells and B cells in vitro will then be quantified. Finally, multiple sensors will be fabricated in arrays to allow simultaneous detection of different species and admix purified proteins or mixtures of cells to evaluate the fidelity of measurement in more complex environments. The third specific aim is analysis of the more complex process of cytokine-regulated antibody production using a multiple array sensor in vitro.

Tirrell, David A | California Institute Of Technology | New Amino Acids For Protein Engineering | GM062523 | 3 years | NIGMS

Abstract: DESCRIPTION: Protein engineering is a powerful tool for design if novel liquid crystal phases, macromolecular surface arrays, reversible hydrogels, and artificial extracellular matrices for use in tissue regeneration and repair. In vivo microbial expression of artificial genes provides a means of preparing such non-natural proteins in high yields. The target structure is encoded into an artificial gene, and the gene is expressed in an appropriate microbial host. However, in vivo protein engineering poses a challenge in that the pool of potential monomers is restricted to the natural proteinogenic amino acids and those analogs that can be activated and charged to transfer RNAs. Tirrell and others have successfully incorporated analogs of methionine, isoleucine, leucine, and phenylalanine through the action of their respective aminoacyl-tRNA synthases. Analogs with olefinic and acetylenic functional groups have been shown to serve as methionine surrogates in bacterial protein synthesis. Incorporation of such functional groups creates important new opportunities for chemical derivatization, extending the range of materials properties that can be designed into protein-based polymers. For example, recent advances in the chemistry of olefin metathesis have led to the development of transition metal carbenes that catalyze efficient cyclization of peptides containing olefinic side chains. The objective of this proposal is to combine fast computational analog screening methods and experiments - both in vivo and in vitro - to find new amino acids for use in protein engineering. This collaboration will lead to fast and efficient discovery of non-natural amino acid analogs with new and useful functionality and will provide a basis for building novel protein-like polymers with desired properties. The computational methods to be used here have already been tested for design of analogs for phenylalanine and will be extended to new substrates for Phe, Met, Ile, Leu, and Val tRNA synthetases.

Torzilli, Peter A | Hospital For Special Surgery | Cartilage Degeneration Following Joint Trauma | AR047656 | 3 years | NIAMS

Abstract: DESCRIPTION (Verbatim from the Applicant): The objective of this application is to study the post-impact response of articular cartilage and subchondral bone to mechanically-induced, subfracture insults that result in irreversible cartilage damage. Such subfracture joint injuries are commonly seen in individuals with acute anterior cruciate ligament (ACL) injury, where radiographs are normal but subchondral microfractures are evident by magnetic resonance (MR) imaging (bone bruises). Recent evidence indicates that, in subfracture joint injury, the initial insult causes irreversible damage to the cartilage, resulting in cartilage thinning indicative of degenerative joint disease. Surprisingly, little or no information exists on the initial damage to the articular cartilage in subfracture joint injuries, nor on the cartilage and subchondral bone post-injury response. We propose the hypothesis that there is a unique threshold of impact which will cause chondrocyte death and collagen fiber rupture, but not subchondral bone microfracture, and that these cell- and matrix-specific injuries will be irreversible and lead to further cartilage degeneration. The specific aim of this proposal is to determine the mechanical conditions (threshold stress and stress rate) and the physical mechanisms causing macrostructural, microstructural and cellular damage in impacted articular cartilage, and to evaluate the post-impact physiological response using an in vivo rabbit model. There are three phases to this study. In Phase I, we will determine the threshold stress and stress rate that causes cell and matrix damage, and the chondrocyte, collagen and subchondral bone response at 0 and 24 hours, and 1 week after impacting viable, mature bovine articular cartilage-subchondral explants. In Phase II, we will scale the threshold parameters from Phase I to the rabbit knee in order to produce three levels of impact injury (below, at and above threshold). Finally, in Phase III, we will study the in vivo post-impact response in the rabbit knee at 1, 2, 4, 8 and 12 weeks. Our hypothesis will be tested at the cellular level by determining the mechanical factors (magnitude and rate effects) causing chondrocyte damage (recoverable vs. permanent, i.e. death), the intra-cellular location of damage (membrane, cytoskeleton, nucleus), and the cell's metabolic response (anabolic vs. catabolic), and at the structural level by determining the spatial location and cause of cell and collagen damage (zone; mechanical vs. catabolic), proteoglycan degradation and loss, subchondral bone damage, and general biomechanical properties. If our preliminary findings (hypotheses) are validated, then individuals sustaining subfracture injury would be predisposed to post-traumatic arthritis, and would dictate that new methodologies would have to be developed for the diagnosis and treatment of these types of subfracture joint impact injuries.

Venugopalan, Vasan | University Of California Irvine | Photon Migration for Measurement of Small Tissue Volumes | CA092063 | 1 years | NCI

Abstract: The overall goal of the proposed research is to develop non-invasive optical technologies for the real-time, quantitative measurement of optical and physiological properties in small tissue volumes. Currently, optical techniques that employ the detection of diffusely transmitted or reflected light are most often used in conjunction with diffusion-based optical transport models to either (a) image centimeter-thick, highly- scattering, heterogeneous tissues with approximately 5mm. spatial resolution or (b) quantify optical and physiological properties of large, highly-scattering, homogeneous tissue volumes (>50 mm[3]). Although such techniques can also be used to measure a volume-averaged impact of localized heterogeneous structures, the current inability to accurately characterize light transport on small length scales (equal to or < 5mm) significantly hampers the possibility of accurately quantifying optical properties in small, well-defined tissue volumes. Here, we propose a comprehensive theoretical, computational, and experimental approach to substantially extend diffusion approximation limits by enabling the assignment and quantification of optical and physiological properties to small localized tissue volumes (approximately 2-50 mm3) of arbitrary albedo. These properties have been shown to be sensitive, quantitative measures of cellular and extracellular morphology and biochemical composition. Such a capability will spur the development of novel, compact optical probes with broad application including early detection of dysplastic transformation of epithelial tissue structure and composition, intraoperative/endoscopic surgical guidance, as well as diagnostic feedback for real-time monitoring and control of photodynamic, hyperthermal, cryogenic, and coagulative therapies. Such probes are also valuable for basic biological studies in artificial tissue and pre-clinical animal models where the spatial scales probed are inherently small. The proposed research aims to fully develop both a novel optical modeling approach to describe light transport on sub-millimeter length scales as well as computational algorithms to determine optical properties from photon migration measurements made in small tissue volumes. These methods will be extensively tested and validated through the experimental measurement and computational processing of light signals that result from propagation through realistic tissue phantoms. We will apply these newly developed photon migration methods, in conjunction with thick-tissue microscopy techniques, to examine artificially- engineered tissue models for normal and dysplastic epithelia. This latter study will allow us to investigate the interrelationships between microscopic tissue morphology and composition and mesoscopic optical absorption, scattering and anisotropy coefficients provided by photon migration methods.

Vigneron, Daniel B | University Of California San Francisco | 3D MR Spectroscopic Imaging Of The Newborn Brain | NS040117 | 5 years | NINDS

Abstract: DESCRIPTION (Verbatim from the Applicant's Abstract): Brain injury in term and preterm neonates is a serious problem. Of the approximately 42,000 infants born yearly in the United States with a birth weight less than 1500 g, approximately 85 percent survive and, of these, 5-10 percent exhibit major motor deficits and another 25-50 percent exhibit developmental and visual difficulties. Hypoxia and ischemia frequently occur during the birth process; however, the amount of brain damage in these patients and the long-term neurologic outcome varies considerably from patient to patient. There is a need, particularly in this group, to identify new clinical diagnostic tools that will improve early prediction of neurodevelopmental abnormalities and therefore allow for pharmacological interventions. The goal of this bioengineering research project is to develop and implement advanced Magnetic Resonance spectroscopic imaging techniques to detect the distribution of metabolite levels throughout the brain of neonates. Studies by ourselves and others have indicated an important role for single voxel MRS in the assessment of the neurologic status of neonates, especially premature infants and those with suspected neonatal hypoxia. However, these techniques provide very limited coverage of the brain and at poor spatial resolution. In this study, we propose to develop and optimize MRSI techniques to provide, for the first time, a study of the 3D distribution of metabolite levels in the newborn brain. This information will define the normal variation in metabolite levels with anatomic location and post-conceptional age. The database of normal MRSI spectra will improve our understanding of brain development and provide a reference for detecting abnormal metabolism in neonatal patients with neurologic damage. Current methods are inaccurate for assessing the cerebral metabolism of newborns. Through this project, we aim to develop a noninvasive metabolic imaging technique to address this important problem.

Vohra, Yogesh K | University Of Alabama At Birmingham | Nanocrystalline Coatings for Dental TMJ Implants | DE013952 | 3 years | NIDCR

Abstract: DESCRIPTION (provided by applicant): The Departments of Physics, Dental Prosthodontics and Biomaterials, and Department of Oral and Maxillofacial Surgery at UAB propose an interdisciplinary program to evaluate nanocrystalline coatings for dental implants. We propose to test the hypothesis that the wear and bone fixation characteristics of TMJ (temporomandibular joint) implant devices can be significantly improved by manipulation of the nanostructure of diamond coatings (adhesion and wear) and hydroxyapatite coatings (fixation). Nanocrystalline diamond coatings with surface roughness of only 5-10 nm will be produced by microwave plasma chemical vapor deposition process with methane/hydrogen/nitrogen species. Our preliminary adhesion and toughness studies have shown no shear failure/loosening of diamond particles up to 150 kg indentation load. Nanocrystalline calcium phosphate (primarily hydroxyapatite) coatings will be deposited by pulsed laser deposition techniques onto the screws and device surfaces in contact with bone. Precise control of chemistry and structure of the thin coating should result in a more optimum surface for fixation with bone. Novel design strategies will be implemented for uniform coating over curved surfaces by rotation and translation of the implant in the activated plasma. Nanostructured -diamond and -hydroxyapatite can be interfaced on the same implant, provided diamond deposition at high temperature is done first followed by a low temperature deposition of hydroxyapatite using pulsed lasers. Nanoindentation studies will be carried out to measure the modulus and hardness of coatings as well as wear testing under simulated dental conditions. In vivo biocompatibility testing of nanostructured diamond and calcium phosphate ceramics will be carried out using standardized model systems, including injection of particulate debris, plus soft and hard tissue evaluations of particulates and implants. The in vivo part will be carried out in collaboration with an oral and maxillofacial clinician/surgeon at UAB, who actively participates in the area of TMJ surgical reconstruction.

Vorp, David A | University Of Pittsburgh At Pittsburgh | Biomechanical Evaluation Of Abdominal Aortic Aneurysm | HL060670 | 4 years | NHLBI

Abstract: DESCRIPTION (Verbatim from Applicant's Abstract): Abdominal aortic aneurysm (AAA) is a focal enlargement of the infrarenal aorta. If left untreated, AAA will gradually expand until rupture; an event that carries a mortality rate of 90 percent and which is ranked as the 13th most common cause of death in the US. Associated with surgical repair of AAA are significant costs and risks to the patient. Thus, it is important to determine when the risk of rupture justifies the risks associated with surgery. The ability to reliably evaluate the susceptibility of a particular AAA to rupture could vastly improve the clinical management of these patients, as there presently exists no such reliable evaluation criterion. AAA rupture occurs when the mechanical stress (i.e., internal forces) acting on the aneurysm wall exceeds its ability to withstand these stresses (i.e., the wall's failure strength). The greater the wall stress to wall strength ratio for a particular AAA, the greater the likelihood of rupture. They therefore believe that a "biomechanics-based approach" would lead to an improved assessment of the propensity for rupture of AAA on a patient-specific basis. Before the decision is made for surgical intervention for an individual patient, the investigators believe that the surgeon should know two things: the mechanical stress acting on that aneurysm and the strength of the AAA wall. They have recently made great strides toward these ends. However, to improve their AAA wall stress estimates the biomechanical behavior of the AAA wall needs to be more carefully and rigorously defined. Therefore, Specific Aim #1 is to refine their current methodology to account for the more realistic, anisotropic biomechanical behavior of AAA. They previously showed that there occurs a significant, 50 percent decrease in strength of the AAA wall versus nonaneurysmal aorta. However, they did not determine spatial variation of AAA wall strength. Specific Aim #2 is to develop a statistically based, multifactorial model to noninvasively estimate spatial variation of AAA wall strength. Specific Aim #3 is to generate the spatial variation of the rupture potential index (RPI) for individual AAA. The RPI is defined as the locally acting wall stress divided by the local wall strength. As the RPI nears a value of 1.0, AAA rupture is imminent. The clinician may therefore inspect the RPI distribution in order to make an evaluation for a particular AAA. This novel, innovative, computer-based noninvasive method would be an important and reliable diagnostic tool to guide the surgeon in decisions for elective repair of AAA, greatly improving the clinical management for those afflicted with this disease.

Zhao, Yunxin | University Of Missouri Columbia | Enhanced Multimedia Telehealth for Hearing Disabilities | DC004340 | 5 years | NIDCD

Abstract: DESCRIPTION (Provided by Applicant): Telemedicine or telehealth has opened a world of specialty health services to persons who may otherwise be unable to access appropriate care. Despite enormous public investment in telehealth initiatives, people who are deaf or hard of hearing encounter serious barriers in accessing current systems because of limited audio quality and limited sign language and lip reading capability. In addition, current videoconferencing systems are hardware-dependent, rendering this technology costly to initiate and upgrade. This project proposes a software driven array of adaptations to current telehealth systems that will facilitate speech and sign language comprehension. The project will achieve three aims: 1) It will enhance language comprehension by developing a real-time voice driven captioning system that automatically transcribes the speech of health care providers for display at both patient and health care provider telehealth sites, 2) It wilI facilitate lip reading and sign language reading by developing a high quality low delay software-only video coding system that can deliver clear motion scenes of lips and hands in synchrony with both speech and captions, and 3) It will evaluate the effectiveness of these technological innovations in facilitating acceptable language comprehension levels in telehealth interactions among persons across the hearing spectrum. While the proposed aims focus on persons with hearing loss, it is expected that the multimedia enhancements to telehealth will significantly benefit persons with intact hearing as well. The effects of the proposed innovations are far-reaching. Medical specialty services including: emergency services, cardiology, psychiatry, which currently use telehealth network systems will become more accessible to deaf and hard of hearing patients, including many elderly persons with hearing loss. The proposed adaptations also will allow for telehealth to support additional services such as speech/language therapy, and cognitive rehabilitation; thus, broadening the array of services available to rural and other underserved populations. Improved access to timely care for deaf and hard of hearing persons will likely improve the health of these populations. In addition, it will reduce the dual isolation of deafness in rural America. The proposed innovations will have notional and worldwide applicability and will spur health-related research in telehealth applications as well as the need of traditionally undeserved populations including deaf, hard of hearing and elderly persons.