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PROJECT TITLE: Biomolecular Analysis Using Liquid Crystals

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute

ABSTRACT

Our BRP is focused on the development of new molecular analysis tools for identifying and validating biological endpoints whereby novel anti-cancer agents can be more accurately and rapidly evaluated regarding their molecular mechanism(s) and clinical relevance. A multi-disciplinary team of researchers with expertise in chemical and biological engineering, chemistry and biochemistry, and the biomolecular and biomedical sciences is developing a bioanalytical approach that integrates advances in the following areas: a) the nano-fabrication of surfaces, b) the development of synthetic and biochemical strategies for the covalent and oriented immobilization of proteins and peptides on surfaces, c) the implementation of liquid crystals as highly sensitive reporters of the presence of proteins captured on surfaces, and d) the investigation of key cell signaling proteins that participate in processes associated with carcinogenesis. Specifically, the analytical characteristics of liquid crystals for reporting the behavior of the well-recognized anti-cancer target, i.e. the EGF receptor, are being compared to conventional analytical methods in a study that will a) rapidly and sensitively assess the levels and activity of wild-type and mutant human EGF receptor in biological samples, b) test the hypothesis that wild-type and oncogenic forms of the EGF receptor exhibit differential inhibitor specificity, and c) assess if agents that potently inhibit EGF-mediated events in vitro will also exhibit a capacity to antagonize EGF receptor expression and/or activity in cell culture. Our studies use the EGF receptor system as a prototype and it is anticipated that the technology can be adapted to other molecular targets. In the long term, these new tools should be useful for the assessment of the molecular mechanisms and consequences of anti-cancer agents, thereby facilitating their research from basic biology through to clinical assessment of efficacy.

STATUS OF RESEARCH AND PARTNERSHIP

Our BRP was initiated in August of 2005. In year 1 of the grant, we have made substantial progress towards the goals of Aim 1 of the proposed research. In particular, we have been successful in using advanced lithographic and nanofabrication tools at the UW Center for Nanotechnology (CNTech) to fabricate dense well-defined periodic structures with lateral feature dimensions (periods from 40 nm to 80 nm) and relief dimensions (2 nm to 10 nm) at the nanoscale. In future studies to be performed under Aim 2, these nanostructured surfaces will be used in combination with liquid crystals to detect binding of proteins such as EGFr to antibodies immobilized on these surfaces. In addition, we have demonstrated the feasibility of key element of methods for the covalent and oriented attachment of peptides and proteins using the Staudinger ligation. This immobilization chemistry will be employed in future research under Aim 1 to immobilize single chain antibodies to EGFR on the surfaces of the nanofabricated substrates.

Perhaps the most exciting development of research in year 1 relates to progress in Aim 2 towards demonstrating detection of EGFR using liquid crystals and nanostructured surfaces. In year 1 research, we have successfully demonstrated that it is possible to combine the use of nanostructured interfaces and

liquid crystals (LCs) to report antibody-mediated capture of EGFr from cell lysates and membrane extracts onto surfaces. Key results obtained Year 1 demonstrated a simple method for detection of EGFr from cell membrane extracts and crude cell lysates. The method does not require matched pairs of antibodies, as is required for surface-based fluorescence assays, nor does it require the complex instrumentation associated with methods such as mass spectroscopy or surface plasmon spectroscopy. Because LCs can be used to image regions of surfaces that have micrometer-dimensions, high sensitivities are theoretically possible using the methods reported in this paper. We calculate the areal density of EGFR molecules giving rise to the response of the LC to be ~103 molecules/μm2. These results and others obtained in year 1 research suggest that liquid crystals and nanostructured surfaces may offer the basis of a broadly useful molecular analysis tool to quantify the activity and expression level of wild-type and mutant human EGF receptors using cell extracts and purified proteins.

As described in the proposal, the majority of tasks to be performed in Aim 3 will be pursued in later years of the grant, employing the methodologies emerging from Aims 1 and 2. In pilot studies conducted in year 1 of the grant, we have successfully demonstrated detection of the activity of EGFr TK inhibitor, AG1478. In short, wild type cells, in the presence of AG1478 and EGF were shown to express EGFr but not phosphorylated EGFr. These results, when combined, demonstrate the feasibility of developing an analytical methodology based on LCs and nanostructured surfaces that would be broadly useful in basic cell biology studies aimed at understanding the effects of receptor mutation on the activity of EGFr TK inhibitors.

ISSUES

None. The technical aims of the research are unchanged.

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PROJECT TITLE: Optimizing Heart and Brain Cooling During Cardiac Arrest

PARTNERS' NAMES AND AFFILIATIONS:
   University of Chicago (Chicago, IL) and Argonne National Laboratory (Argonne, IL)

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT
   The overall objectives of the project are to develop improved more rapid and better controlled methods for cooling human victims of cardiac arrest as well as others medical conditions involving possible ischemia damage; to ultimately improve survival rates by using hypothermic protection in these patients. The project utilizes a swine model of cardiac arrest and cooling for the development of novel human compatible "ice-slurry" micro-particulate coolants. Specific Aims include: I. Engineering and developing optimal micro-particulate ice slurries and associated delivery equipment for biologic use. II: Optimizing brain and heart cooling using saline or perfluorocarbon slurries. III. Describing and minimizing adverse effects of slurry coolants on tissues. IV: Testing the effects of bridging hypothermia on cardiac arrest survival.

STATUS OF RESEARCH AND PARTNERSHIP
   In this third year of the grant, our bioengineering team of physicians, biological scientist and engineering scientist from the University of Chicago and Argonne National Laboratory have substantially advanced our understanding of: a) micro-particulate slurry use as a human coolant, b) implementation protocols associated with the different modes of using the coolant, and c) the equipment needed for slurry production and delivery. The year's primary focus has been on conducting the experiments directed at Specific Aims I (creating a better microparticulate slurry for use), II (optimizing the cooling techniques with the saline ice slurry), and III (performing additional short term survival tests to evaluate potential adverse effects on tissue of slurry). Our general model utilizes 40-50kg swine that are anesthetized and instrumented for multiple measurements of temperature, blood flow, and hemodynamics. In overview under Specific Aims I, II, & III, we are continuing to develop an optimal slurry coolant method that can be quickly delivered by an EMS rescuer or by in-hospital care givers that is both safe and effective and provides protection against ischemia. We have begun work on Specific Aim IV - Testing the effects of rapid cooling during swine cardiac arrest.
   We have continued our studies to further develop the slurry cooling method using GI slurry delivery and cooling through the stomach which functions as an in-body heat exchanger. This mode of cooling is believed to be the easiest to administer in the field and by EMS rescuers. We have quantified the effectiveness of using dosages of 20 to 50 cc slurry /kg animal weight and have examined animals 7 days post cooling for possible adverse effects from GI ice-slurry cooling. We have modified standard GI tubing to avoid slurry plugging. This delivery tube has a thermocouple for measuring stomach temperature during cooling and we have succeeded in delivering 49% by wgt. ice slurry as measured by calorimetery immediately before delivery. We are now able to achieve 2 to 4 C brain cooling by the use of these dosages. A series of 10 day survival tests were conducted for ascertaining whether the slurry would cause any adverse GI tissue effects. Slurries of temperatures from -1 to -6 C were used. These studies showed no adverse tissue effects from using slurry in the range that we propose. This work has led to new intellectual property regarding instrumentation required in the use of slurry.
   This year we spent considerable effort on developing a swine full cardiac arrest model that includes survival after 10 minutes of untreated ventricular fibrillation plus an additional 10 minutes of CPR prior to defibrillation. This is a very long arrest time and highly mortal. We have now demonstrated our ability to provide cooling and to upgrade multiple new instruments for use in quantifying brain protective effects

of hypothermia. New technology includes the routine of microspheres for multiple organ tissue flow calculation, improved hemodyanic solid state sensors, as well a carotid blood flow measurement during arrest and resuscitation. We have demonstrated a significant improvement in coronary perfusion pressures with use of the inspiratory threshold value, as well as hemodynamic changes with the administration of saline during resuscitation. In the coming rear we will conduct full cardiac arrest scenario tests involving: animal preparation/instrumentation, inducement of cardiac arrest, inducement of rapid cooling with IV or GI supplied slurry (and as a reference cold saline) for varying periods of down-time with chest compressions, followed by defib, warm up, animal recovery, and adverse effects assessment.

During this year we have also made further improvements in our ability to achieve higher ice loadings. We are now capable of producing reliably 49% ice slurry, as determined by calorimetry, which can be reliably delivered through the modified GI and other relatively large diameter specially designed delivery tubes. With the high ice content slurries being produced it has been discovered that all commonly used devices such as catheters, ET , GI and other medical tubes are prone to ice particle plugging because they were designed for delivery of single phase liquids. Improved delivery tubing is required for optimal administration of slurry. This work has led to filing of a patent application on designing medical delivery tubing and injector tips for various modes of slurry cooling that have been developed over the first three years of this grant. See the below list of six patents granted and applied for on medical ice slurry cooling technology which have resulted from this grant to date.

We have developed a novel method to make sterile slurry. Conducting the full scenario cardiac arrest tests of item 2 will require the use of sterile slurry in the protocol. To accomplish this, we have developed a fully integrated multi-step ice slurry production machine for making human operating room quality sterile slurry. This machine has generated an invention report that has been filed and will be used in the remaining two years of this grant for conducting survival tests in swine. This development of sterile slurry has been a major challenge to the use of IV slurry; progress in this area is a major accomplishment of the project.

In order to more fully explore the applicability of cooling other organs with slurry we have broadened our work to involve exploring the use of slurry cooling for cooling the kidney during minimally invasive laparoscopic surgery for inducing protection from ischemia damage during blood vessel clamp-off. We have successfully delivered ice slurry through a specially designed tube which interfaces with conventional laparoscopic ports. The slurry is used to coat the external surface of the kidney and in 10 minutes we have demonstrated dropping the temperature by more than 15 C. This use of slurry cooling has the potential for facilitating urology surgery procedures done laparoscopicaly by greatly extending the surgical window under clamp-off from 30 to more than 90 minutes. A patent application has been filled for this new laparoscopic cooling procedure using slurry.

ISSUES

All results to date confirm the advantages of ice-slurry over similar volumes of ice cold saline. Faster cooling and less volume is used with slurry compared to saline. The intravenous route is extremely rapid compared with all other routes of administration (and with other more conventional methods of cooling). The GI route also appears to be an important route for cooling. It has the advantage that it is less invasive, easy to administer and yields predictable cooling. It could be possible for paramedics or emergency personnel to rapidly initiate cooling using the GI route and to achieve target temperature in under 30 minutes. This would be a significant improvement over current techniques that require 3-4 hours. In terms of eventual implementation, the GI route may also be easier for regulatory clearances. Finally, other uses of slurry cooling being demonstrated for surgical applications show great promise of expanding the application of this method of medical cooling. Our efforts in the coming year (4 th year) will be to test our slurry hypothermia inducement methods using a full scenario cardiac arrest survival model to confirm survival benefit (Specific Aim IV). In addition, we will further develop our slurry production machine to provide sterile slurry which is easily controlled and administered for a variety of cooling modes. We will also continue to develop computational models for predicting slurry cooling effectiveness and explore other applications of slurry. We will also begin to explore the combined effects of cooling using multiple routes of administration. Plans for renewal of funding and measures to continue support for the work are additionally underway.

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PROJECT TITLE: Breast CT Scanner for Earlier Cancer Detection

PARTNERS' NAMES AND AFFILIATIONS:
    Main institution: University of California, Davis (Sacramento, CA)
    University of California, San Diego (San Diego, CA)
    Varian Imaging Systems (East Palo Alto, CA)
    Duke University (Durham, NC)

    Consultants: University of California, San Francisco, Stanford University, Hahneman University (Philadelphia, PA), University of Arizona, Tucson.

GRANTING NIH INSTITUTE/CENTER: This award was originally funded by the National Cancer Institute; however, it was subsequently transferred to the National Institute of Biomedical Imaging and Bioengineering.

ABSTRACT
    Breast cancer is a disease with high incidence in the U.S. and elsewhere, and population-level methods of fighting this disease are aimed primarily on screening, using mammography for early detection. The median size of breast cancer found using mammography is approximately 11 mm. Based on extensive preliminary studies involving computer simulations, physical measurements, and cadaver breast imaging, we have found that breast CT may be able to routinely detect much smaller breast tumors, in the 3 to 5 mm range. Importantly, the radiation dose of breast CT performed at 80 kVp was found in detailed studies to be comparable to that of mammography. It is not possible to image the breast alone on a live women using a clinical CT scanner. Therefore, in this Bioengineering Research Partnership proposal, we have teamed with scientists from around the country to design, build, and test a CT scanner designed to image the breast. A team comprised of medical physicists, physicians, mechanical and electrical engineers, and breast cancer advocates will collaborate on the design of the breast CT scanner. Cone beam flat panel technology will be used to produce a scanner capable of 10 second breast scanning, and the scanner development will also include a breast immobilization system (acrylic cylinders), a breast CT table, a fast reconstruction computer, and a computer workstation customized for efficient viewing breast CT images. The scanner will be built, tested, and optimized at UC Davis over a period of 3 years involving 9 specific aims. After the breast CT scanner is tested in a brief phase I trial (2 specific aims), it will be moved to the breast imaging clinic for a phase II trial where approximately 120 women will be imaged (4 specific aims). This phase II trial will evaluate the efficacy of breast CT for the early detection of breast cancer in a group of women likely to have breast cancer (BIRADS 4 & 5). Additionally, the breast image data will be studied for its utility in automating the analysis of the normal breast architecture, and for computerized cancer detection. In year 5 of the proposed research, two specific aims utilize the breast CT data and corresponding mammography images (on ~240 breasts) to evaluate the ideal observer performance and human (mammographer) detection performance attributes of the breast CT scanner.
    At the end of the proposed research involving 17 specific aims, the potential of breast CT will have been evaluated both qualitatively and quantitatively. A tested, high quality prototype breast CT scanner

would be ready to be enlisted in a phase III trial (beyond the scope of this proposed research), if further testing is warranted. Performance data acquired in the present study would allow the proper design (power, etc.) of a phase III trial. If breast CT lives up to its enormous potential based on initial imaging, breast cancer would be detectable far before metastasis occurs – for example, a 3 mm tumor contains only 2% of the cell count of an 11 mm lesion, and a 5 mm lesion contains only 9% of the cell count. Based on a 100 day volume doubling time, detection of a 5 mm lesion would lead to 0.93 year earlier detection, and routine detection of 3 mm lesions would result in 1.5 year earlier detection over mammography. Surgical removal of early cancers will effectively result in cure for the majority of women screened using this technology. While breast CT would probably improve cancer detection in all women, some women may have risk factors (dense breasts, genetic markers, etc.) that particularly warrant screening using breast CT. The Phase II trial will shed more light on this issue.

STATUS OF RESEARCH AND PARTNERSHIP

The research is going extremely well and we are now studying the efficacy of breast CT in a clinical trial. We have completed a Phase I trial of 10 healthy volunteers, and have begun Phase II evaluation. To date, we have scanned a total of 24 patients (Phase I and II). As we accrue patient images on our first prototype breast CT scanner, we are also fabricating the second prototype breast CT scanner, and this system will be completed we think by September 2005. We are also study a wide variety of issues associated with breast CT during the time in which the scanner is not being used for patient imaging, including patient dosimetry, phantom development, image quality, x-ray scattered radiation and its effect on images, beam hardening, cone beam algorithm advancements, etc.

ISSUES

We have no major issues or problems to report. We have maintained strong collaborative relationship with the partners within the same time zone (all in California), one partner's participation has been compromised by personal health issues. We are developing additional collaborators, which we feel will become partners upon competitive renewal – this include Dr. Andrew Maidment at University of Pennsylvania and Dr. Robert Nishikawa at the University of Chicago.

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PROJECT TITLE: An Organotypic Model of Traumatic Brain Injury

PARTNERS' NAMES AND AFFILIATIONS:

Dr. Simon, Dr. Xiong, Dr. Lusardi (RS Dow Neurobiology Labs), Dr. Rochefort (Oregon State University)

GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke

ABSTRACT

The past decade has witnessed intense scientific activity to investigate molecular mechanisms of traumatic brain injury, driven by overwhelming evidence that neuroprotection by pharmacological inhibition of apoptosis has the potential to dramatically reduce the effects of brain trauma. Key requisite for the systematic investigation of neuroprotective agents is an accurately characterized, clinically relevant in vitro brain injury model. Despite this obvious need, the ability to deliver such defined, realistic trauma to specimens in vitro lags far behind the sophistication of molecular and biochemical assays used to measure the response. This Bioengineering Research Partnership brings together neurobiologists and bioengineering scientists to developed an in vitro brain injury model, which subjects organotypic brain cultures to acceleration-induced shear injury. In this model, organotypic brain cultures realistically model the in vivo apparent heterogeneous cell population in a three-dimensional cellular matrix, while inertial acceleration-induced shear strain delivers a scalable, defined, and clinically relevant mechanical insult.

We hypothesized that our acceleration model of organotypic brain cultures can realistically reproduce traumatic brain injury, in which the delivered shear strain magnitude can be quantified on a cellular level. Exercising our model, we determine cell type specific injury vulnerability and biological injury cascades in response to a defined mechanical insult.

To date, we have completed a formal experimental characterization of our novel brain injury system, including assessment of the delivered angular acceleration magnitude and determination of the constitutive properties of the organotypic culture (Aim 1). The resulting experimental source data were required to formulate a validated analytical model that allows computational simulation of the shear injury throughout the brain specimen for any point in time during the primary mechanical insult (Aim 2). Subsequent to this rigorous system characterization, we exercised the brain injury model to establish dose/response histories (Aim 3). Over the past year, we implemented the in vitro brain injury system to investigate cell-type specific injury susceptibility, time-history of the biological injury cascade, and effects of hypoxic brain injury secondary to the mechanical insult (Aim 4). We furthermore advanced the existing model toward a 3rd-generation in vitro brain injury system, which will be more user-friendly, robust, and allows for higher through-put experiments in six-well culture plates.

Upon successful completion, the results of this integrative research approach will yield a well-characterized, scalable, reproducible and clinically relevant brain injury model. Considering the vast interest in therapeutic interventions now under development aimed at inhibiting the cascade of secondary effects of primarily mechanical brain injuries, our organotypic trauma model will directly address the rapidly increasing demand for a well-characterized experimental system to deliver a clinically relevant traumatic insult.

STATUS OF RESEARCH AND PARTNERSHIP

In the third year of our research partnership, we were able to employ the brain injury model for characterization of sublethal injury, and we implemented, characterized, and utilized the 3rd generation device.

Model Advancement: The 3rd generation model was implemented and fully characterized. In this model, organotypic cultures in a six-well acceleration module are subjected to a uni-axial, inertia-driven shear strain induced elongation by rapid acceleration during linear impact. Impact severity and magnitude of shear elongation are controlled by modulating the impact velocity. This advanced model allows for reproducible and timely completion of TBI experiments with up to six culture wells in less than one minute. Transient shear elongation of organotypic cultures was recorded with a high-speed camera at 40,000 frames/second. This allowed quantification of the shear displacement magnitude and duration, and residual shear displacement. As such, the advanced TBI model enables simulation of well characterized, graded, and reproducible mechanotrauma in vitro in a robust, high-throughput setting.

In this advanced TBI model, we characterized the strain in hippocampal slices during a graded range of accelerations, recording both the dynamic deformation and any residual deformation with respect to the original, unstrained slice. Total elongation, the sum of both elastic and plastic deformation was measured during the dynamic deformation phase of the experiment. The plastic component was measured as the residual deformation in the static image following acceleration. Significant hippocampal cell death was measured only in cultures following accelerations with significant plastic (unrecovered) elongation. Following accelerations resulting in no measurable residual strain, no hippocampal cell death was measured, even as late as 72 hours following the insult.

Model Employment: Clinically, hypoxia is a significant factor complicating patient outcome following TBI. Using oxygen-glucose deprivation (OGD) as a model of hypoxia, we showed that sublethal acceleration sensitizes the cultures to a subsequent sublethal OGD. This sensitization is calcium dependent, and begins to wane by 6 hours following acceleration. Further studies measured the field-evoked post synaptic potentials (fEPSP) in the CA1 region of the hippocampus, showing increased excitability in the accelerated cultures compared to the control cultures, suggesting a synaptic contribution for the sensitization to OGD.

ISSUES

The collaboration within the BRP has progressed smoothly. Frequent interaction between the engineering group (led by Dr. Bottlang) and neurobiology group (led by Dr. Simon) have produced an injury model that is both well characterized and highly useful for studying the biological effects of traumatic brain injury. Manuscripts are in preparation to describe the details and characterization of the engineering model and the functional changes measured in the hippocampal slice culture following a purely inertial, sublethal deformation.

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PROJECT TITLE: Dynamic Signal Processing Analyses of Neural Plasticity

PARTNERS' NAMES AND AFFILIATIONS:

Dr. Wendy A. Suzuki (Center for Neural Science, New York University), Dr. Matthew A. Wilson (Department of Brain and Cognitive Science, Massachusetts Institute of Technology)

GRANTING NIH INSTITUTE/CENTER: National Institute on Drug Abuse

ABSTRACT

In response to PAR-02-010, Bioengineering Research Partnerships, we propose to form a research partnership between a statistician (Dr. Emery N. Brown of Massachusetts General Hospital, Partnership Director), two neuroscience experimentalists (Dr. Matthew A. Wilson of the Massachusetts Institute of Technology and Dr. Wendy Suzuki of New York University) and a control engineer (Dr. Victor Solo of the University of New South Wales) to develop a systems engineering approach to understanding neural plasticity. The area of bioengineering research will be the development of neural signal processing algorithms combining the theory of point processes and adaptive estimation to study neural plasticity during learning in both the rodent and monkey medial temporal lobe regions. The experimental investigations will systematically study the dynamics of neural activity within the hippocampus and adjacent medial temporal lobe structures (entorhinal, perirhinal and parahippocampal cortices) in rats, genetically altered mice, and primates. These experimental studies will provide the basis for a focused investigation that develops neural signal processing methods appropriate for dynamic analysis of multiple simultaneously re corded neural spike trains. The algorithms we develop will be used to analyze the data collected in the experimental studies proposed in this investigation. The close collaboration between the experimentalists and the quantitative scientists will ensure that the methods designed are appropriate for the data collected. The objectives of this partnership are to provide a careful quantitative description of neural plasticity and how it relates to learning, memory formation and behavior, and to develop broadly applicable signal processing tools for analyzing the dynamic behavior of neural ensembles.

STATUS OF RESEARCH AND PARTNERSHIP

During Years 1 to 3 of the parent application we have made strong progress on all three specific aims.

Specific Aim 1: Dynamic Analysis of Information Encoding within the Hippocampus (Matthews A. Wilson, MIT).

We are now completing our initial experiments involving the simultaneous recording of CA1, CA3, and DG neurons during exposure to familiar and novel environments and have identified novel receptive field properties in the DG (Specific Aim 1A-B) (Nakazawa et al. 2003). Work has been completed and is being prepared for publication on the PSD-95 KO line that displays learning deficits along with enhanced LTP of hippocampal synapses. Our analysis of spatial receptive field properties and dynamics in these animals has established a novel phenotype that relates to receptive field shape characteristics and dynamics that was proposed to be studied in specific aims A-C. Behavioral studies and hippocampal recordings are being completed on a new line of DG-specific NMDAR-KO animals (Specific Aim 1C). Work is in progress collecting similar data from mice with CA3-restricted genetic deletion of NMDA receptors (Specific Aim 1C).

Specific Aim 2: Dynamic Analysis of Information Encoding Within the Hippocampus and Adjacent Regions of the Medial Temporal Lobe (Wendy A. Suzuki, NYU).

Our major goal has been to understand the neural correlates of associative learning and memory across the different structures of the medial temporal lobe in macaque monkeys. We have made progress in three main areas. First, we now have a detailed understanding of the kinds of dynamic learning signals present in both the hippocampus and adjacent perirhinal cortex during the performance of an associative learning task (location-scene association task) (Specific Aim 2A-B). Both areas signal learning with striking changes in their stimulus-selective response properties and both areas can signal new learning both before, at the same time and slightly after behavioral learning is expressed (Wirth et al. 2003). The major difference between the two areas appears to be the time course within the trial that the learning signal is seen. The perirhinal cortex signals information early in the trial during the visual stimulus period of the task. In contrast, the hippocampus signals learning later in the trial during the memory delay period. Second, we have evidence that both areas also signal selective information about very well-learned associations (Specific Aim 2A-B). Well-learned information is signaled by a significantly more selective response compared to novel information and this long-term memory signal is present in both areas (Yanike et al, 2004). Third, in collaboration with Dr. Craig Stark, we have recently shown that the same striking learning signals observed in the hippocampus and perirhinal cortex in monkeys is also present in BOLD fMRI responses throughout the human medial temporal lobe (Law et al. 2005). These findings, taken together suggest that multiple areas across the medial temporal lobe signal new associative learning in both monkeys and humans.

Specific Aim 3: Dynamic Signal Processing Methods for the Analysis of Neural Plasticity (Brown, MGH/HMS; Solo, U. Mich.).

We described a new paradigm for estimating the state space models from point process observations (Smith and Brown (2003) that served as the basis for the dynamic estimation algorithm we used in Wirth et al. (2003) to estimate the behavioral learning curves and relate it to changes in neural spiking activity of neurons in the monkey hippocampus. We have developed a general paradigm for point process adaptive filtering (Eden et al., 2004) and neural spike train decoding (Barbieri et al., 2004). The results extend our previous work, defines for point process observations, the analogs of the steepest descent recursive least squares and the Kalman filter algorithms for continuous-valued processes and significantly enhances our ability to track neural plasticity on a millisecond time-scale. We have demonstrated that hippocampal neurons form place representation in 5 to 6 minutes of exposure to a novel environment. However, behaviorally it took substantially longer for the animals to recognize the new environment as familiar (Frank et al. 2004). We extended our point process framework for analyzing neural spike trains by developing a GLM model that allows us to relate spiking history, neural ensemble and covariate activity to the spiking propensity of individual neurons (Truccolo et al., 2005). We developed an efficient algorithm for fitting multivariate point process models of neural spiking activity (Okatan et al. 2005) in order to estimate simultaneously the effects of members of a group of neurons on each other. We developed new definitions learning, algorithms to characterize it, and showed that our approach characterizes learning more accurately than current methods (Smith et al., 2004; Smith et al 2005).

ISSUES

We have not encountered any issues that have impeded the progress of our work.

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PROJECT TITLE: FES and Biomechanics: Treating Movement Disorders

PARTNERS' NAMES AND AFFILIATIONS:

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GRANTING NIH INSTITUTE/CENTER: National Institute of Child Health and Human Development

ABSTRACT

This multi-investigator project combines resources from four professors of Mechanical Engineering and three professors of Physical Therapy through our newly organized Center for Biomedical Engineering Research at the University of Delaware. The five-year goal of this project is to assist patients with CNS dysfunction to produce improved walking patterns through a combination of functional electrical stimulation (FES), robotic-assistive training and biomechanical modeling. In the first phase of this project, which is described in this proposal, the focus will be on individuals with stroke exhibiting hemiparetic leg impairment. The technique should be generalizable to a variety of neurological impairments. The movements for these individuals will be improved or "optimized" in four ways: Nonrisk--Maximize postural stability, Injury--Minimize musculoskeletal injury (e.g., arthritis) during movement, Cosmesis--Develop a more natural looking gait, and Energy--Minimize metabolic energy consumption during movement. The NICE optimization protocol will be realized through musculoskeletal modeling, robotic assistance, functional electrical stimulation, and neuromuscular training. The specific task we will study will be partial body weight suspension gait on a treadmill. The organization of this project has been divided into 3 distinct aims, which may be summarized as follows. Aim 1: Identify impairments in the locomotor patterns of the lower extremity in patients with hemiparetic stroke and create a paradigm to optimize the movement patterns ("NICE" optimization). This will be accomplished through biomechanical modeling using gait analysis and electromyographic data. Aim 2: Develop the methods and equipment "NICE" rehabilitation system) necessary to implements the "NICE" optimization of locomotion in patients with stroke. We will achieve this through the use of a robotic device and an electrical stimulation system. Aim 3: Test the feasibility of the use of the "NICE" rehabilitation system in patients with hemiparetic stroke and make adjustments to the system based on the patient trials. Our ten-year goal is to produce a portable (wearable) FES system to assist patients with CNS dysfunction in the production of coordinated movements.

STATUS OF RESEARCH AND PARTNERSHIP

The Partnership is doing very well. This project involves the coordination of teams working on three subjects: biomechanical modeling, FES, and robotics.

Biomechanical modeling: We have developed a biomechanical model of the ankle to use for this project. This model characterizes the morphology and force generating capacity of the musculature spanning the ankle. We have developed techniques to obtain parameters for the model from MRI and ultrasound to make it subject-specific. The model also allows us to estimate muscle forces from EMG signals during dynamics tasks. We have used this model to predict muscle activation patterns during gait

and to determine how they could be modified in patients with strokes to produce an improved gait pattern, which can then be input to an FES protocol. Further development of the stroke model will be our focus next year.

FES: The major activities for the past year have been the continued development of the hardware and software interfaces needed to allow the feedback from the robot to be used to produce real time modification of the stimulation pulse duration and frequency to match the desired muscle force outputs. In addition, we have continued to test and develop our mathematical models that allow us to predict force responses to electrical stimulation. Results show that our model predicted very well the peak force and force-time integrals produced in response to stimulation trains of different frequencies (10-80 Hz) and patterns (constant, doublet and variable-frequency trains). The successful testing of our mathematical model on ankle muscles of individuals with stroke supports the model's potential use in predicting suitable stimulation parameters when controlling ankle motion during FES.

Robotics: We have developed a passive gravity-balanced leg orthoses for the human leg that can fully or partially balance the leg over its range of motion. These orthoses are being used in clinical studies of subjects with stroke to modify their walking patterns. An active gravity balancing rehabilitation machine was fabricated, based on our experience with passive gravity balancing device. This active device has all the features of the passive device, such as four degrees-of-freedom for the trunk, two degrees-of -freedom at the hip joint and one degree-of-freedom at the knee joint. Also, this device has actuators at the hip and knee joints to help initiate a desired gait to the user. An innovative ankle-foot orthosis (AFO) was also designed and fabricated that allows two degrees-of-freedom motion to the ankle, while serving to maintain proper foot position for subjects. The prototype AFO will introduce greater functionality over currently marketed devices by means of its inversion-eversion degree-of-freedom in addition to flexion/extension.

ISSUES

There are no technical, administrative, or programmatic issues that have arisen in regards to our partnership.

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PROJECT TITLE: Combined Digital X-Ray and Ultrasound Breast Imaging

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute

ABSTRACT
The first goal is to leverage recent technological advances in mammography and ultrasound by designing and evaluating a common compression paddle platform to interface the two imaging systems. The platform will enable co-registered, semiautomated acquisition of breast images with the two modalities to mitigate the dependency on operator and interpreter skills. Initially, the diagnostic results should be equivalent to those obtainable by experts with the two modalities independently. The second goal is to leverage this platform to develop and compare advanced modes, including 3D x-ray tomosynthesis, US (ultrasound) color flow and elasticity imaging.

STATUS OF RESEARCH AND PARTNERSHIP
a. **Specific Aims:** Goals of the project and partnership arrangements have not changed since revisions preceding the notice of grant award.

b. **Studies and Results:**

Digital X-ray/Ultrasound Breast Imaging System: A full field digital mammography (FFDM) and digital tomosynthesis system has been developed by the GE research group. GE had advice and support from the U of M group as well as others, such as Capt. Jerry Thomas of the Uniformed Services University of the Health Sciences, and funding from this BRP and Capt. Thomas's Office of Naval Research grant MDA905-00-10041. The tomosynthesis system is the first of its kind, a second generation system with much more rigid base and mechanics, x-ray tube and electronics to allow faster image acquisition with almost twice as many view angles. With a new, more sensitive detector, the dose is the same as the first generation system at MGH, one tomo volume = ¾ the dose of a two-view mammogram set. The existing ultrasound scanning system has been refurbished and modified to work with the new tomo/digital mammography unit in a way that will not require removing the ultrasound transducer holder during x-rays. When delivered in July, this will be our first complete system that is available purely for this research.
Advanced Imaging Modes: Major efforts have continued in development of advanced ultrasound and x-ray imaging modes which promise to provide substantially different information from that of the FFDM and US images of the core combined system.
Nonlinear Elasticity Imaging: Elasticity images are created with the combined, or a standalone, system by increasing compressing for 2 seconds to create 10% maximum strain changes. Off-line, the acquired RF images are correlated using phase-sensitive, 2D speckle tracking techniques. They are further processed to produce high CNR throughout the image. Initial human imaging demonstrated elasticity imaging's ability to distinguish between fatty and glandular tissue, as well as malignant and surrounding tissues at 1-3% strains.

Vascularity Analysis: Remaining hardware timing and storage issues related to cardiac-gated Doppler imaging were addressed. The work is still in early stages of assessing our ability to quantify local abnormalities or in blood volume. To date, 35 subjects have participated in automated Doppler scans. Of these, five were scanned at multiple times (4) and/or under varying compression levels (4). A scheme was developed to automatically measure changes in relative blood volume for co-registered sequential Doppler scans. For a 7% decrease in paddle separation, mean blood volume was relatively stable, +18% to -30%. Changes in flow with compression may aid in detecting vascular anomalies from cancer.

Digital Tomosynthesis Mammography: Preliminary tests were performed by co-investigators from the U of M in early July, 2005 using a first generation GE Senographe 2000D clinical digital mammography system that was modified to acquire tomosynthesis projections and with the second generation tomosynthesis system being readied at GE Global Research for delivery to the U of M. Following in vitro and human testing of the new system, we should be ready to begin the main clinical trials promised in this project.

Advanced Image Processing: Image-based registration: Using image grayscale information alone, in-house image-based registration software (MIAMIFuse) was used to co-register image volumes under different conditions, and both hand selected and Doppler-segmented fiducial points served as markers for registration accuracy. Sequential scans could be accurately co-registered despite variation in patient positioning between scans (as measured by inter-scan mean pixel displacement, MPD).

Multimodality CAD: Using cases containing mammograms and the corresponding 3D US cases from an earlier system, we developed algorithms to extract and merge computer-extracted features for the computer-aided differentiation of malignant and benign masses. The classifier that combined the two modalities exhibited a higher accuracy than those using each modality separately. Two radiologists were significantly more accurate when they read cases with CAD ($p=0.05$).

CAD of Breast Masses on Digital Tomosynthesis Mammograms (DTMs): A preliminary mass detection program was developed using a data set of 26 DTM cases acquired by a GE prototype system at MGH. The mass system achieved similar, slightly better results than those obtained with higher dose, conventional mammo-graphy. We also studied the dependence of the CAD system performance on image quality of reconstructed DTMs.

Clinical Studies: Design evaluation and imaging technique studies have been performed on 40 normal volunteers and patients under an IRB protocol. Additional subjects were studied before and after neoadjuvant chemotherapy of their breast cancer with this system as part of a separate grant. The first 10 cyst cases scanned at consistent settings to allow quantitative as well as visual assessment of in vivo image quality were analyzed quantitatively, as well as subjectively by experienced breast imaging radiologists. Image quality is quite acceptable through the 1 to 2.5 mm. We have continued progress in stabilizing the breast and achieving good coupling between essentially all of the breast and the compression paddle for optimum ultrasound and x-ray imaging.

ISSUES

X-ray and ultrasound breast imaging provide substantially different information and are used together clinically. Combining them should reduce time requirements for trained staff and reduce uncertainties about co-identification of lesions. While some of the advanced modes have been researched independently, no cohesive studies demonstrating relative clinical benefits of these modes have been performed and each is being done somewhat better than previously given the well constrained tissues. All should eventually facilitate the introduction of ultrasound into routine breast screening of patients with dense breasts.

Close coordination between partners UofM and GE GRL and good faith efforts and decisions have kept us on track for having an excellent set of features operational for the prospective clinical trials to begin soon after the new tomo/FFDM/advanced US system are delivered in July, 2005. There are severe cost constraints caused by substantial cuts at the time of grant award. We continue to devote substantial effort to developing, maintaining and working with versions of the complex Logiq 9 ultrasound system software and image fusion software which are unique for this 3D project, while trying to obtain and maintain compatibility with state of the art capabilities of the scanner in important features such as multiview, color flow, trapezoidal, speckle reduction and (for another study) contrast agent imaging.

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PROJECT TITLE: Light-Directed Synthesis of Genes and Other Biomolecules

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GRANTING NIH INSTITUTE/CENTER: National Institutes of Health, Public Health Service,
U.S. Department of Health and Human Services

ABSTRACT:
a. **Specific Aims**
     The following are the specific aims for year one of the BRP project as funded after the budget was
reduced on recommendation of the Scientific Review Group and Council.
     1.   Bioinformatics:
          a.   Develop and test algorithms for the design of the subsequences and gene assembly process.
     2.   Chemistry:
          a.   Develop DNA synthesis and release chemistry using the safety-catch photolabile linker (SCPL)
          b.   Apply SCPL protecting groups for use in light-directed combinatorial synthesis of small
               molecule microarrays with the Maskless Array Synthesizer (MAS).
     3.   Biochemistry:
          a.   Develop and optimize the gene assembly process.
          b.   Develop error correction using MutS-based consensus shuffling.
     4.   Engineering:
          a.   Develop next generation Automated Gene Synthesizer (AGS) instrument.
     5.   Applications:
          a.   Development of chemistry to enable surface invader probe arrays
B.   Studies and Results
     In May 2005 we have repeatedly demonstrated assembly of 500bp DNA fragments synthesized on a
single Chip. This proves the validity of our approach, and opens the way to synthesis of longer constructs
as promised in the proposed activity.
     1.   Bioinformatics
          The focus for the first year of the gene assembly project has been the optimization of the light
          directed DNA synthesis process in order to produce oligos with a minimum of errors and the
          release and assembly of theses oligos into 500bp building blocks for long DNA sequences.
          During this process development phase the existing in house software Gene Design Assistant
          (GDA I) has been sufficient for dividing target DNA sequence in sets of oligos for assembly. In
          the next year features will be added to the software to automatically add and test oligo
          amplification primers for the AACED process, eliminate interfering DNA secondary structures
          and design variable length oligos based on the Tm of the assembly overlaps. Due to budget cuts
          we are currently under staffed in this area.
     2.   Chemistry
          •   DNA synthesis and release using the safety-catch photolabile linker (SCPL): A first
               generation safety-catch photolabile linker (SCPL) has been synthesized and spatial
               photorelease of DNA demonstrated after acid activation of the safety catch [1]. Current
               efforts are focusing on the design and synthesis of a linker, which will function at longer
               wavelengths. Several model compounds have been synthesized and tested and a second-
               generation linker is currently being synthesized.
          •   Use of safety-catch photolabile protecting groups in light directed combinatorial chemistry:
               Proofs of principle experiments have demonstrated the use of safety-catch photolabile
               protecting groups for light directed combichem [2]. Current efforts are focused on the synthesis
               of a small molecule library based on statins, which are known Aspartly protease inhibitors.
     3.   Biochemistry
          •   Optimization of the gene assembly process: The initial protocol for light directed oligo
               synthesis on the AGS system was based on one developed by NimbleGen Systems for the
               production of high-density oligonucleotide microarrays for gene expression studies. While

these oligo chips perform well in DNA hybridization applications sequencing of oligos cleaved from chips produced in house and from NimbleGen showed that only 9–25% of the oligos were of the desired sequence. Poor oligo quality produced unreliable gene assembly reactions and unacceptably high error rates in the assembled DNA fragments. In order to improve oligo quality we evaluated a number of changes to the synthesis protocol such as capping incomplete strands, blocking synthesis in the borders around the DNA features, optimization of the light and fluidic cycles and reduction in the number of oxidation steps. Each protocol change was assayed by PCR amplification of the eluted oligos (AACED [3]) followed by cloning and sequencing multiple samples from each chip. These changes to the oligo synthesis process have increased the quality of the synthesized oligos from 9% to 71% perfect sequence.

- Advances in error filtering: We have developed a MutS based method for eliminating errors in long dsDNA fragments [4]. The method termed Consensus Scuffling fragments long error containing DNA segments with an endonuclease then uses MutS to remove the smaller error containing segments [4, 5]. The resulting error free DNA is then reassembled via PCR to form functional DNA. In an initial demonstration, the consensus shuffling method was applied to 10 nonfunctional (non-glowing) GFP clones after the first round of consensus shuffling 30% of the clones glowed and 82% glowed after two rounds. Further optimization of the consensus shuffling method should allow the assembly of essentially error free DNA.

4. *Engineering*
- Development of next generation AGS system: During the past year, significant progress has been made improving the AGS tool design. These improvements have been focused on reducing stray light effects, improving light uniformity and resolution across the exposure field. As a result, transition to a more user-friendly, optically optimized AGS tool design from the current prototype platform is expected this summer.
- Development of an AGS fluid delivery system: Initially all Maskless Array Synthesizer based oligo chip synthesis systems used ABI Expedite 8909 DNA synthesizers to deliver the phosphoramidite synthesis reagents. These older tools have severe shortcomings. An improved delivery system has been designed and built and is currently being tested. We are confident that this new system, which uses rotary fluid valves for stream selection and a syringe pump for precise volume control, will be in full production use by the end of the summer.
- Development of a capillary oligo synthesis system: One possibility for the next generation AGS system is greatly simplified design composed of a capillary packed with quartz micro-spheres where the light activated DNA synthesis chemistry is driven by a series of UV LEDs.. In preliminary studies 25-mer mixed base oligos have been synthesized inside a treated glass capillary tube using UV LEDs as the light source and an Expedite for fluid delivery. Hybridization results show conclusively that DNA is only produced in the illuminated areas, and that the correct sequences were grown. Additionally, a mismatch experiment was run, and hybridization showed a clear difference between perfect-match and mismatch oligos.

5. *Applications*
- Development of chemistry to enable surface invader probe arrays: An important component of the Bioengineering Research Partnership (BRP) is real world applications of the technology developed under the grant. The Smith group is developing a DNA microarray based approach for large-scale analysis of SNPs based on highly reliable Invader cleavage assay [6-9]. This technology will allow the simultaneous testing for thousands of SNPs from unamplified genomic DNA. The AGS systems high-density light-directed oligos synthesis is ideally suited for the synthesis of the necessary DNA arrays. The focus of the first year of work has been the evaluation of substrates that are compatible with both the oligo synthesis process and the invader assay conditions. Substrates made of glass, glassy carbon, carbon nanotubes and diamond films have been evaluated for oligo synthesis and compatibility with fluorescent detection. Thus far the glassy carbon substrate has been the most promising producing the best fluorescent signal. During the next year, we will expand on these results and determine which material performs best (most stable, least non-specific cleavage, etc.) in surface invasive cleavage reactions.

STATUS OF RESEARCH AND PARTNERSHIP
See abstract.

ISSUES
See abstract.

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PROJECT TITLE: Optical Biopsy Using MEMS Technology

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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute

ABSTRACT

The broad, long term objective of the proposed research is to develop a noninvasive system for optical biopsy using microelectromechanical system (MEMS) technology. We propose to combine the advances in biomedical imaging and MEMS technology to develop a high speed, endoscopic functional optical coherence tomography (OCT) with a miniaturized probe for early diagnosis of lesions and tumors in gastrointestinal (GI), respiratory, and urogenital tracts.

The specific aims of this work are to: (1) design and develop a high speed, fiber optic based high resolution functional OCT system for endoscopic imaging of in vivo tissue structure and blood flow dynamics in GI tracts, and investigate and develop hardware systems and imaging processing algorithms for speckle noise minimization and imaging enhancement (Chen); (2) design and develop scanning probes with silicon MEMS technology (Tian); (3) design and develop scanning probes with polymer MEMS technology (Li and Bachman); (4) integrate MEMS probe with OCT system and perform in vitro and in vivo testing (Chen, Tien, Li, Bachman, Chang); and (5) investigate the applications of MEMS based endoscopic OCT for early diagnosis of lesions and tumors in GI tracts (Chang and Chen). This is a collaborative project that involves PI and Co-PIs with expertise in biomedical optics, silicon and polymer MEMS technology, and endoscopic imaging. The scanning probes developed using MEMS technology have the advantage that they are compact, robust, low cost, low power requirement, and high speed. In addition, lateral resolution of the current endoscopic OCT that uses axial scanning followed by lateral scanning is limited by the focal depth of the probe beam. The high scanning rate of the probe made with MEMS technology offers the potential to increase lateral resolution by performing lateral scanning first in order to maintain the beam waist at the zero optical path length. Furthermore, a scanning probe fabricated with MEMS technology has the potential to provide three-dimensional imaging of tissue structure and physiology with high imaging speed. Finally, the scanning probe technology developed in this proposal can also be used for endoscopic confocal and two-photon imaging.

STATUS OF RESEARCH AND PARTNERSHIP

In the third year of this project, significant progress has been made in the advancement of functional OCT technology and endoscopic 2-D MEMS endoscopic OCT probes.

We have developed a high-resolution second harmonic optical coherence tomography (SH-OCT) system that significantly enhances OCT imaging m at the second harmonic wave centerμcontrast. An axial resolution of 4.2 wavelength of 400 nm has been achieved. Because the SH-OCT system uses the second harmonic generation signals that strongly depend on the orientation, polarization and local symmetry properties of chiral molecules, this technique provides unique contrast enhancement to

conventional optical coherence tomography. The system is applied to image biological tissues of the rat-tail tendon. Highly organized collagen fibrils in the rat-tail tendon can be visualized in recorded images.

We have developed a three-dimensional (3-D) endoscopic OCT system based on a dual axis MEMS mirror. The diameter of MEMS mirror was 1.2 mm and both axes were capable of scanning up to 20° (optical) with excellent linearity. The two axis MEMS mirror was packaged in a machined acrylic endoscopic housing which provided mechanical protection, electrical interconnects and optical alignment of the MEMS device to a focusing GRIN lens. The endoscopic MEMS probe was integrated and tested with a fiber-based time domain (TD) and Fourier domain (FD) OCT systems. By means of 2-axis lateral scanning combined with an axial scan, volume images were obtained at a rate of 3 frames/s and 7 frames/s respectively. In the initial investigations, in vivo 3-D OCT images of finger as well as images of animals such as healthy rabbit trachea, normal and cancerous regions of hamster cheek pouch tissue, and GI tract of pig animal model were obtained. These images clearly delineated important features and tissue structure.

The partnership is functioning very well. Investigators regularly visit each other's laboratories, hold bi-monthly joint group meetings, and their students utilize both laboratories for their research.

ISSUES

The original focus of the partnership is the development of endoscopic OCT using MEMS based probes for cancer diagnosis in the GI tract. One issue we face is that the technology we developed also attracts a lot of clinicians from other specialties that would like to use the device. Currently, we have one system set up in the UCI Medical Center that has been used for imaging cancers in larynx and up airway. However, it is difficult to accommodate most of these requests with only one system developed from this grant.

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PROJECT TITLE: Uropathogen Detection Using DNA Biosensors

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT
Urinary tract infection (UTI) is the most common urological disease in the United States and is a major cause of patient morbidity and health-care expenditure. This Bioengineering Research Partnership proposal involves development and testing of an automated system for the genotypic detection and species-specific identification of uropathogens within a time frame (30 minutes from sample collection to readout) that would enable point-of-care diagnosis and treatment. This approach would fundamentally alter the management of UTI and potentially other infectious diseases. Engineering studies have resulted in development of a sample processing system that enables concentration and lysis of uropathogens on a polycarbonate filter membrane. Our corporate collaborator, GeneFluidics, has developed a novel electrochemical sensor array chip that we have functionalized with a panel of oligonucleotide probes designed to detect and identify the 16s rRNA of uropathogens. The sensitivity of the sensor has been further enhanced through improvements in the bacterial lysis strategy and in the design of the capture- and detector-probes. The lower limit of the sensitivity of the target derived from raw bacterial lysates without nucleic acid purification or amplification was 20,000 bacteria/ml. During year three we achieved an important milestone, namely the successful application of the 'UTI chip' to clinical urine specimens. A clinical study was performed involving 89 blinded clinical urine specimens demonstrating 100% sensitivity for detection and identification of Gram-negative uropathogens. Major goals for years four and five of the project are to integrate the UTI chip with the sample processing fluidics component to create a self-contained, automated prototype urosensor device and to validate the performance of the urosensor device in a clinical study of patients at risk of UTI comparing urosensor results with clinical microbiology studies and clinical correlates of UTI.

STATUS OF RESEARCH AND PARTNERSHIP
Project Organization. We are continuing to utilize the organization structure that was put into place during the first year of the project, including monthly research meetings, monthly administrative meetings, and posting of electronic reports on our website.
Microfluidics. Dr. Chih-Ming Ho's laboratory has successfully developed a filtration system that is effective in concentrating uropathogens in clinical urine specimens from patients with urinary tract infection. The filtration system uses a polycarbonate filter membrane housed in an acrylic plastic chamber that is pressurized to drive filtration. Bacterial lysis buffer is added to the material captured on the filter surface yielding a filtrate containing target nucleic acids that can be directly detected by our electrochemical sensor. The filtration system is effective in the concentration of E. coli nucleic acids, yielding a 3- to 4-fold increase in signal using the GeneFluidics electrochemical sensor compared to unfiltered specimens. The performance of the filtration system can satisfy the design requirement for the sample preparation system.

Detection of Uropathogens in Clinical Specimens. Improvements in the performance and consistency of the GeneFluidics microfabricated 16-sensor array chips has allowed experiments to continue to be carried out on a daily basis using the sensor array reader (potentiostat) that was designed and fabricated by GeneFluidics and delivered to Dr. Haake's laboratory at the UCLA/VA Medical Center during year two of the project. Each sensor in the array consisted of three single-layer gold electrodes—working, reference, and auxiliary. The working electrodes were treated with alkanethiolate self-assembled monolayers and functionalized with a library of biotinylated capture probes specific for clinically relevant bacterial urinary pathogens including E. coli, P. mirabilis, P. aeruginosa, Enterococcus spp., and Klebsiella-Enterobacter group. Unlabelled 16S rRNA target derived from single-step bacterial lysis hybridized to both the capture probe on the sensor surface and a second, fluorescein-modified detector probe. Detection of the hybridized targets was achieved through binding of a horseradish peroxidase (HRP)-conjugated anti-fluorescein monoclonal Fab fragment. Amperometric measurement of the catalyzed HRP reaction was obtained at fixed potential and correlated with bacterial concentration. Species-specific detection of uropathogenic bacteria in culture, inoculated urine, and infected clinical urine samples was achieved within 40 minutes. The lower limit of the sensitivity of the target derived from raw bacterial lysates without nucleic acid purification or amplification was 2×10^4 bacteria/ml, or 20 femtomolar 16S rRNA.

Determinants of Electrochemical Signal Intensity. We examined the determinants of signal intensity for detection of uropathogens using the electrochemical sensor array. A 'universal' lysis system was developed that was effective in releasing target nucleic acids from both Gram-positive and -negative uropathogens. Optimal lysis was achieved in two five-minute steps: Treatment with a mixture of Triton X-100 and lysozyme followed by sodium hydroxide. Studies were performed to determine the effects of probe length, fluorescein modification position, and distance between capture and detector probe hybridization sites. 40-mer oligonucleotides with 5'-biotin and 3'-fluorescein modifications demonstrated improved signal compared with shorter or longer oligonucleotides, indicating that there is an optimal distance from the sensor surface for binding of the Fab-HRP complex. The hypothesis of an optimal distance from the sensor surface was consistent with improved signal intensity using 3'- compared with 5'-fluorescein modification of the detector probe, since the 3'-fluorescein modification is oriented away from the sensor surface. Additional improvements in signal intensity were achieved by eliminating the gap between the capture- and detector-probe hybridization sites on 16S rRNA. The combined effects of 3'-fluorescein modification and hybridization site gap elimination led to a 20-fold increase in sensitivity of the electrochemical sensor for detection of Enterococcus species. Finally, the feasibility of a detector probe cocktail was demonstrated. These discoveries result in enhanced detection sensitivity and simplified sample preparation, greatly reducing the design complexity of the microfluidics component when the sensor array is eventually integrated into an automated device.

Clinical Study. Most importantly of all, we have successfully applied the 'UTI chip' to clinical urine specimens, which is, to our knowledge, the first detection of bacterial pathogens in human specimens using a microfabricated electrochemical sensor array. A clinical study was performed to compare electrochemical sensor assays with standard clinical microbiology results for detection and identification of uropathogens in clinical urine specimens. We tested the analytic validity of the electrochemical sensor assays on 89 blinded clinical specimens. A preliminary algorithm for analysis of the sensor data indicates that the electrochemical sensor has 100% sensitivity for detection and 98% sensitivity for identification of Gram-negative uropathogens in specimens containing no more than one organism. Sensitivity was reduced for Gram-positive organisms due to the resistance of these organisms to alkaline lysis. An improved lysis method applicable to both Gram-positive and –negative bacteria was subsequently developed (see above). A second clinical study is planned for year four that would involve a larger number of specimens from 200 patients involving improved probes targeting additional organisms. A detailed statistical analysis of the data will be conducted in collaboration with Dr. Elliot Landaw.

ISSUES

None. The BRP granting mechanism has greatly facilitated this multidisciplinary effort, which cuts across traditional academic and institutional structures.

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PROJECT TITLE: Molecular Analysis of Breast Cancer

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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute

ABSTRACT

Many women with small, node-negative breast cancers are essentially overtreated. For example, most Stage I breast cancers are treated with both local and systemic therapies but ~ 80% are effectively cured with local interventions alone. Separating these patients from the ~20% who recur, irrespective of their treatment, remains problematic. Consequently, the development of novel methods that can more accurately predict for a nonrecurrent vs. recurrent phenotype is a major priority. We address this issue in our response to PA-02-010, for which we have established an imaginative and integrated Bioengineering Research Partnership comprising three research teams (Bioengineering & Biostatistics; Clinical & Pathology; Microarray & Molecular Analysis) from three local universities (Georgetown University, The Catholic University of America, The Arlington Research Institute of Virginia Tech) and the University of Edinburgh (Scotland, UK). We will apply expression microarray and tissue array technologies and powerful new data analysis algorithms to define the gene expression profiles of 600 invasive breast tumors (Stages I-III). Our multidisciplinary teams will use these molecular profiles and established prognostic factors to build artificial intelligence-based classifiers and multivariate models that accurately predict those patients with nonmetastatic disease (especially Stage I) who will/will not recur. In the long terms, the genes in this classifier and the classifier's algorithms will be used to build custom diagnostic arrays and software for routine clinical use.

Hypotheses: We hypothesize that differences in the gene expression profiles of tumors determine outcome (recurrence) in patients with nonmetastatic disease. We also hypothesize that computational bioinformatics can discover these differences and use this knowledge to build classifiers that predict each patient's prognosis (especially in Stage I disease).

Aim 1: We will perform gene expression analysis on breast needle biopsies of 600 invasive, nonmetastatic breast tumors.

Aim 2: We will build an integrated data processing and management system for data acquisition and retrieval, to support the data analysis algorithms to be optimized and applied in Aim 3.

Aim 3: We will optimize and apply novel pattern recognition and information visualization technologies, recognizing the high dimensional nature of the data, to discover and validate gene subsets that separate recurrent from nonrecurrent tumors. We will integrate advanced artificial intelligence algorithms and biostatistical models to build predictive classifiers that can more accurately define cancer phenotypes and predict clinical outcomes.

Aim 4: We will use tissue arrays (multiple cores from archival tissues arrayed on glass slides) to validate and optimize the performance of these classifiers in a retrospective prognostic study of human breast tumors.

STATUS OF RESEARCH AND PARTNERSHIP

We have made substantial progress in our work to develop further methods to explore and solve very high dimensional data sets. A key component of our bioengineering studies is validation of the algorithms in existing data sets and comparison against existing technologies. We have successfully compared VISDA for cluster modeling, discovery, and visualization with bottom-up hierarchical clustering and non-model based self-organizing map algorithms (programmed in MatLab) and verified by the ground truth (these are existing/published microarray datasets where outcomes are known). Performance measures were obtained via randomized 10-fold cross-validation and can be considered generalizable. VISDA outperformed both existing technologies, with at least a 90% detection accuracy, a lower estimation bias of cluster parameters (at least a 10% improvement), and lower estimation variation of cluster centers (at least 10% improvement), averaged over all trials.

A second goal was to develop a machine classifier that could predict the behavior of an unseen case belonging to one of the targeted cancer subtypes, together with iterative selection-nonlinear regression cross-phenotype normalization (ISNR-CPN) and comprehensive diagnostic gene selection methods. We have successfully demonstrated the superior performance of optimized multilayer perceptrons (oMLP) for both multiple and binary class prediction, as compared with conventional MLP (cMLP). Using the same number of input genes, oMLP consistently outperforms cMLP, with higher prediction accuracy (at least 20% improvement), faster convergence rate (at least 20% improvement), and larger Az value (at least 10% improvement via the area under the Receiver Operating Characteristics analysis), averaged over all trials. Performance was obtained via randomized three/five-fold cross-validation and be considered generalizable. We have also demonstrated comparative performance by oMLP and multiclass support vector machine (MSVM) with three kernel functions, in terms of both prediction accuracy and Az value (only for binary cases).

ISSUES

Our biggest problem has been accrual of sufficient numbers of informative cases but we have recently recruited another research nurse and accural has substantially increased. We have had no other major issues and our Bioengineering Research Group continues to find working with investigators from very different backgrounds (clinicians, medical oncologists, engineers, computer scientists) exciting, rewarding, and highly productive.

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PROJECT TITLE: 3D Imaging and Computer Modeling of the Respiratory Tract

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT
The respiratory tract is one of the main interfaces between the body and the environment. As such, the respiratory tract can become a target for a broad range of airborne environmental agents contributing to an expansive array of human diseases. To improve our ability to predict the dosimetry and thus the consequences of airborne pollutants (gases, vapors, particulates or atmospheric releases of chemical/biological weapons) or drugs intentionally administered by inhalation for normal or potentially sensitive populations, 3-dimensional (3D), biologically based models of the respiratory tracts of animals and humans will be developed. The overall specific aims of this BRP are to: (1) develop and apply magnetic resonance imaging and fluorescent microsphere techniques to determine the dynamic, 3D structural and functional properties of the respiratory tract; (2) determine the 3D cellular organization and metabolic capacity; (3) develop and extend software and computational capabilities for 3D modeling and upscaling techniques for cellular-to-organ model integration; (4) develop a normalized atlas of rat geometries with explicit measures of variability; (5) conduct in vivo gas exchange and particulate dosimetry studies for model validation and identification of model uncertainties; and (6) provide a web-based "respiratory physiome" platform for dissemination and training of researchers and clinicians in the use of imaging and annotated model databases. Five projects were designed to provide the necessary data on the dynamic structure and function of the respiratory system for the development and validation of the computational models. To support these five projects, three technology development cores were established in advanced imaging, computation, and database/modeling access and training for external users. A fourth core serves as an administrative interface and provides statistical support among the participating institutions and projects.

STATUS OF RESEARCH AND PARTNERSHIP
Due to the reliance of several projects on the technology cores, a concerted effort was placed on their rapid start-up during the first year of this BRP. As a result, Dr. Brian Saam and colleagues from the University of Utah developed a state-of-the-art hyperpolarized 3He gas generating facility at PNNL along with a MR-compatible ventilator for maintaining anesthetized rodents, delivering hyperpolarized gas, and synchronizing MR imaging with breathing activity. The respiratory physiome and public outreach core led by Dr. Jim Bassingthwaighte and colleagues at UW and CIIT-CHR developed several simplified

models to support the gas exchange and particulate dosimetry projects. Drs. Kevin Minard, PNNL, and Tom Robertson, UW, also evaluated several particles ranging from 40 nm to 1 micron for their combined fluorescent and magnetic properties. These will be integral in year two when they will be used in conjunction with magnetic resonance imaging (MRI) and cryomicrotoming to evaluate regional ventilation and perfusion. Comparisons between in vivo MRI results and data acquired with fluorescent microspheres using the cryomicrotome will be critical not only to evaluate non-invasive imaging protocols for visualizing structure function relationships but also for validating computational models of particulate dosimetry carried out by Drs. Timchalk, Einstein, and Minard at PNNL. As a part of that effort, Dr. Minard, PNNL, has already achieved one of the specific aims of the BRP by successfully achieving quantitative magnetic particle detection in airway phantoms by 3He/MR imaging. Additional work on 1H MR imaging is underway to complement the gas imaging for live animal particle dosimetry. To begin establishing a data base for airway structures 4 rat nasal airways have been imaged with MR and Dr. Plopper's group at UC Davis prepared 12 lung casts. Four casts have been imaged so far for inclusion in the normalized airway geometry atlas for rats (RAtlas) being developed by Dr. Hoffman and colleagues at the University of Iowa. Drs. Plopper and Buckpitt, UC Davis, also began modifying their whole mount approaches for defining cell populations by immunohistochemistry and proteomics methods for defining proteins involved in oxidant protection and chemical metabolism. Dr. Minard has also begun live animal imaging with 3He for obtaining additional airway geometry data as well as evaluating different methods for creating magnetization grids for studying tissue mechanics. Drs. Minard, Jacob and Einstein at PNNL have also successfully matched gas flow MR imaging with CFD model simulations in simple airway phantoms - a necessary step in model validation in live animals. Dr. Hlastala and colleagues at UW have also refined their methods for measuring gas or vapor exchange in rats and humans that, along with particle dosimetry studies will further serve to validate the 3D models of the respiratory system.

ISSUES
None.

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PROJECT TITLE: Absorption Mechanisms of Peptide/Protein Drugs Via Lung

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT
Oral administration of newly bioengineered peptide/protein drugs is often ineffective due to degradation by gastric and intestinal digestive enzymes. As an alternative route for systemic absorption of such protein/ peptide drugs, transpulmonary delivery has shown considerable potential. In this proposal, our long-term goals are to elucidate the mechanisms for absorption of various classes of peptide/protein drugs across the alveolar epithelium (that affords a vast surface area and relatively low protease activity). Although pulmonary delivery of protein / peptide drugs in animal studies has been shown to yield much better bioavailability compared to oral delivery, absorption mechanisms and pathways are mostly undefined to date. Many bioengineering-related issues are associated with pulmonary drug delivery, including formulation of specific drugs, modes of delivery and transport mechanisms. Of these, we will investigate various transport mechanisms that facilitate absorption of peptide/protein drugs across alveolar epithelium, using cultured rat and human alveolar epithelial cell monolayers as in vitro models, and will extend key in vitro findings to in vivo rat lung studies. Model proteins/peptides to be explored range from oligopeptides to proteins of biological importance (e.g., calcitonin, insulin, granulocyte-colony stimulating factor, and human growth hormone). Our research plan is subdivided into three major projects: i) investigate transcellular transport mechanisms (e.g., fluid-phase, receptor-mediated and/or adsorptive transcytosis) for absorption of model drugs across the alveolar epithelial barrier, ii) elucidate strategies for enhancement of alveolar epithelial absorption of protein/peptide drugs via paracellular and /or transcellular routes (e.g., transient alteration of barrier properties), and iii) study enhanced receptor-mediated transcytosis of macromolecule drugs (e.g., conjugation with transferrin in the presence of trans-Golgi disruptors). The collaborative investigation of pulmonary protein/peptide drug absorption among several different biomedical research laboratories, utilizing different experimental approaches spanning cell biology to bioengineering/physiology, promises success in providing pertinent information on advancing practical approaches to pulmonary drug delivery.

STATUS OF RESEARCH AND PARTNERSHIP
Projects 1 and 2 (Delineate mechanisms of transcellular absorption of protein drugs by fluid-phase, receptor-mediated and/or adsorptive transcytosis across the alveolar epithelial barrier / Determine enhancement of alveolar epithelial absorption of protein/peptide drugs by modulating transcytotic and/or paracellular routes):
We have been studying the mechanisms underlying IgG transport across primary cultured rat alveolar epithelial cell monolayers (RAECM) grown on tissue culture-treated polycarbonate filters. IgG appears to be net absorbed across the RAECM via a saturable process, suggesting that alveolar epithelial IgG transport may be mediated by specific receptors recognizing IgG. Since FcRn in neonatal intestine mediates net

absorption of IgG, we designed primers based on published sequence of rat FcRn gene and performed RT-PCR of RNA obtained from primary RAECM on d5. Analysis indicated that sequence of the RT-PCR product is identical to that predicted from rat sequence. Treatment of RAECM with 100 nM dexamethasone for two days (from d5 to d7 of primary RAECM) led to ~50% reduction in FcRn mRNA by Northern analysis, concomitant with a decrease in IgG flux in the apical-to-basolateral (A-B), but not opposite, direction. These results are consistent with the hypothesis that net absorption of IgG across rat alveolar epithelium occurs via FcRn-mediated transcytosis which appears to be regulable by glucocorticoids.

In order to explore transpulmonary IgG transport in vivo, male (~400g) Sprague-Dawley rats were anaesthetized with ketamine and xylazine ip and intratracheally administered 0.02 mg of biotinylated rat IgG (biot-rIgG) dissolved in 0.2 mL phosphate-buffered saline (PBS, pH 7.4), in the presence and absence of 0.22 mg of unlabelled rat Fc, using an intratracheal microspray device. Blood samples were subsequently collected at regular intervals from the tail vein or at 18h by heart puncture and analyzed by enzyme-linked immunosorbent assay using streptavidin-coated microplates with peroxidase-conjugated donkey anti-rat F(ab')2 to detect biot-rIgG. Results showed that [biot-rIgG] in the circulation peaked at 18h. Co-administration of excess unlabelled rat Fc significantly inhibited biot-rIgG absorption, with ~33 ng biot-rIgG/mL serum observed at 18h for rats receiving biot-rIgG alone and ~13 ng biot-rIgG/mL serum observed at 18h for rats receiving excess unlabelled Fc. Pharmacokinetic analysis using samples taken at 0.5, 1, 2, 4, 8, 18, and 24h post-administration yielded an absolute bioavailability (compared to intravenous bolus dose) of intratracheally administered biot-rIgG of ~ 5%, which decreased to ~2% in the presence of unlabelled excess rat Fc. These data suggest that exogenously administered IgG is transported from airspaces to blood in rat lungs in vivo, and that IgG absorption is dependent on Fc-mediated processes (most likely FcRn-mediated transcytosis).

Project 3 (Elucidate the mechanisms underlying enhanced protein drug absorption via transferrin receptor-mediated transcytosis): We studied the effects of non-biodegradable cell penetrating peptides (e.g., Tat peptide and heptaarginine) to determine potential as delivery vectors for macromolecules including proteins and peptides. These cell permeating peptides, due to their ability to penetrate cell membranes rapidly and efficiently, may help transport other peptide and protein drugs across primary RAECM. Cell monolayers were utilized for transport studies between d5 and d7 in culture. Tat peptide and heptaarginine were radiolabelled with 125I according to the chloramine-T method. Unidirectional fluxes in the A-B direction were estimated from appearance of radiolabeled peptide in the basolateral compartment following apical dosing as a function of concentration and time. The amount of peptide recycling into the apical or basolateral compartment was monitored at 0, 0.25, 0.5, 1, 2, 3, 4, 24 and 48h following apical dosing and incubation at 37˚C for 1h. Results indicate that both Tat and heptaarginine show a linear concentration dependent increase in A-B transport across RAECM within the range of 1-20uM. At 0.01 mM, A-B transport of these peptides exhibit linear time dependence up to 4h. A 1h pulse followed by 48h chase showed that the peptides recycle back into the apical compartment within 4h, with a lesser but detectable amount transported to basolateral fluid. These preliminary data suggest that Tat peptide and hepta-d-arginine have potential to be used as vectors to deliver drugs across the alveolar epithelium.

We characterized factor(s) in conditioned media from RAECM that we found to be involved in increased transport of various peptides across RAECM. Primary RAECM on d6 were dosed with radiolabeled peptide in serum free media (SFM), serum media (SM), conditioned media from type I-like pneumocytes (CMI) or conditioned media from type II-like pneumocytes (CMII). At the end of the 2h incubation, basolateral media were collected and the amount of peptide transport was used as a biological assessment for the effect of the various dosing media. 3H-mannitol was used as a marker for paracellular transport and horseradish peroxidase (HRP) for fluid-phase endocytosis. Results show that CMII increased the transport of calcitonin and insulin by 50-80% when compared to that of SFM, SM and CMI. The activity of CMII was retained when precipitated with ammonium sulphate or after centrifugation in 50 KDa molecular weight cut-off Centricon. However, activity of CMII was significantly decreased when heated at 80oC for 0.25h. CMII did not affect transport of 3H-mannitol or HRP, but its effect on peptide transport was decreased when incubated at 4oC rather than 37oC. Conditioned media from type II-like rat alveolar epithelial cells appear to contain (a) factor(s) which is (are) heat sensitive and greater than 50 KDa which facilitate(s) transport of peptides across RAECM.

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PROJECT TITLE: Spatiotemporal Brain Imaging: Microscopic and Systems Level

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT

The advent of non-invasive imaging methods such as functional magnetic resonance imaging (fMRI) has made it possible to obtain spatial maps of hemodynamic "activation" in the human brain under a variety of conditions. However, the indirect and poorly understood nature of the coupling between these hemodynamic signals and the underlying neuronal activity has greatly limited the interpretability of neuroimaging results in terms of the underlying biophysics and cellular organization of the brain at the microscopic scale. The overall goal of this project is to develop an integrated suite of technologies to bridge this critical gap. To this end, we have beeen working in parallel on improving the spatial and temporal domains of MRI (Aim1) and optical imaging (Aim2) technologies, and on applying these developments to study neurovascular coupling in the somatosensory cortex (Aim3). Finally, during the past year, we have commenced application of newly developed technological tools to clinically-related experiments (Aim4). Below is the summary of the progress on each of the aims during the funding year 2004/2005.

STATUS OF RESEARCH AND PARTNERSHIP

Aim 1: improve fMRI spatial resolution. Bring MRI to the spatial level where columnar and laminar structures can be studied and compared to invasive measurements, requires increased detection efficiency, addressed by the RF coil development. Phased arrays of small surface coils extend the high sensitivity detection of small surface coils to sub¬stantially larger regions, including bilateral coverage of the brain. The phased array extends the coverage and sensitivity of surface coils by simultaneously receiving the MR signals from multiple small independent coils, each designed to optimize the SNR in a small region adjacent to the coil. During the past year we have developed an 8-channel phased-array RF coil for non-human primate imaging at 3T and used this coil to acquire high SNR data in anesthetized macaques. Our 8-channel phased-array coil consists of seven coils, which are arranged symmetrically around the head covering the frontal lobe, the temporal lobe, and the occipital lobe. An additional coil is mounted horizontally on top. Using this coil we were able to combine the multiple receiver data digitally in a way that independently optimizes the SNR for each pixel in the combined image.

Aim 2: improve the spatiotemporal resolution of optical imaging. Depth resolution is a limitation of the optical methods primarily used to date. In 2003/2004 progress report we described a new method laminar optical tomography (LOT) that has been developed in our group from purely a concept, to a demonstrated practical technique with a true depth resolution. During the past year we have applied LOT to image brain functional activation (see Aim3). In parallel, we have been developing an alternative

methodology, optical coherence tomography (OCT) that also provides depth resolution and can complement LOT due to its sensitivity to light scattering rather than light absorption. Simultaneous OCT and video microscopy imaging performed in the rat somatosensory cortex proved sensitivity of the OCT method to light scattering changes following stimulus-evoked increase in neuronal activity. The dominant signal increased over the 4 seconds following forepaw electrical stimulus and then required ~5 seconds to recover to baseline. The shape of the OCT response correlated well with video microscopy suggesting that this OCT signal may reflect changes in red blood cell density, although neuronal swelling can not be ruled out at this point. Localized regions of strong signal increases where identified that are characteristic of blood vessels. In addition, smaller signal changes were observed In the parenchyma.

Aim 3: apply the new technology to image functional activation. Over the past year we have applied a new method laminar optical tomography (LOT), that has been developed in our group in the funding year 2003/2004, for depth-resolved study of hemodynamic activation in rat somatosensory cortex. Using LOT we were able to image the cortex in 3D to depths of ~2mm with 100-200 micron resolution, making multiple dual-wavelength measurements of rat somatosensory cortex. 3D evolution of cortical hemodynamic activation (oxy-, deoxy-and total hemoglobin changes) was computed from LOT data. Moreover, we were able to reliably extract vascular compartment-specific hemodynamic signals. First, by using the temporal behavior of each compartment as its unique signature, we identified all the 3D spatial components of each involved vascular compartment. Second, using vascular architecture, as determined from two-photon microscopy images of vascular casts, we validated the time-courses extracted as being genuinely originating only from the separate compartments. The good correspondence between the components' locations and the true vascular architecture implies that the extracted functional timecourses indeed also represent the evolution of changes in the three compartments. Features include smaller volume changes, and significantly later onset times in veins, compared to arteries and capillaries. Arterial components show larger volume changes, and much earlier returns to baseline than capillary and venous compartments. Reliable separation of the temporal evolution of the functional response in each compartment allows interpretation of hemodynamic response in terms of the input and output functions to the capillary bed that might correspond closely to the underlying neuronal activity. In addition, these results can be used to genuinely evaluate the accuracy of established mathematical models of functional hemodynamics.

Aim 4: apply new technology in established neuropathological models. During the past year we have begun to employ the imaging technology developed in preceding aims to study animal models of migraine and stroke, in experimental rat and nonhuman primate models. These studies are linked in part by our study of an underlying pathophysiological mechanism - cortical spreading depression (SD). In the rat we have performed concurrent diffusion MRI and diffuse optical measurements during global ischemia. The global ischemia resulted in a rapid drop in blood volume (i.e. total hemoglobin), and oxygen saturation, occurring coincidently with decreased diffusion coefficient (ADC) as a result of widespread anoxic cell membrane depolarization. Water diffusion anisotropy (FA) also increased in the cortex, in agreement to previous reports after stroke both in humans and animal models. Furthermore, we obtained first results detecting cortical spreading depression (CSD) after stroke in nonhuman primates. We used an endovascular model of stroke in macaques, developed recently at MGH. Serial diffusion scans acquired during the stroke induction showed a rapid decrease in ADC in the lesion core and propagating transient ADC decreases in all slices from several regions on the lesion periphery. The ADC transients propagated across normal brain are a rate of about 5mm/min. These transients are peri-infarct depolarizations (PIDs) which are waves of spreading depressions triggered by the initial ischemic event, and are believed to be a mechanism of lesion growth in stroke. These results are consistent with electrophysiological and MRI studies in rodent stroke models.

ISSUES
None.

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PROJECT TITLE: Cell and Molecular Studies in Cardiovascular Engineering

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT
This is a multi-investigator proposal from a single campus that addresses fundamental bioengineering mechanisms in cardiovascular cells and their preclinical application in vivo and ex vivo. Our approach is to understand the cell and molecular mechanisms by which the local physical and chemical environment regulates cardiovascular cell and tissue physiology and to combine this with testing and implementation of engineering principles in tissues and experimental animals. This is particularly important in the cardiovascular system where the biomechanical, structural, and chemical environments are spatially complex. The program addresses both hypothesis-driven and design-driven experimental approaches in varying proportion. The BRP investigators, a mix of biomedically-trained and engineering-trained faculty, share a strong commitment to interdisciplinary research and represent a community of multidisciplinary scholars. Most of the program is physically located at Penn's Institute for Medicine and Engineering (IME), which was established to connect Medical School and Engineering School scientists working at the interface between biomedicine and the engineering, physical, and computational sciences.

STATUS OF RESEARCH AND PARTNERSHIP
- The BRP has an integrative partnership with Childrens Hospital of Philadelphia (CHOP) on the same campus.
- Minority Supplements awarded to graduate students Amanda Lawrence and Fitzroy Byfield.
- An association with the BRP program of Shu Chien (UCSD) was established in 2004 (one meeting so far in Philadelphia, Oct 2004)

Progress Year 4: Substantial progress in all aspects of the program in year 4 (7/04-6/05) resulted in 43 peer-reviewed papers of work supported by the BRP and work was presented at major meetings.Of note is that each year the productivity of this program has increased, reflecting the broad nature of the investigations linked by common themes. The work is disseminated in leading journals in the biomedical, bioengineering, general (e.g., Nature, Science) and specialist fields.
Among several important highlights (lead investigator/s in parenthesis):

1. at the cellular and subcellular levels both in vitro and in situ studies related to mechanotransduction, cytoskeleton, ion channels, membrane biophysics, integrin signaling/ECM, morphogenesis, cell phenotype:
   • Membrane cholesterol-actin interactions in mechanotransduction (Levitan)
   • Imaging Live Cells under Mechanical Stress (Davies)
   • Forced distension of proteins including chemistry on a single protein during unfolding and probe microscopy to measure adsorption and ensemble analysis (Discher)
   • The of lateral cell-cell border location and extracellular/transmembrane domains in PECAM/CD31 mechanosensation (Davies)
   • Neutrophil string formation: hydrodynamic thresholding and cellular deformation during cell collisions (Diamond)
   • Functional expression and molecular identification of mechano-responsive Kir ion channels in endothelium (Levitan/Davies)
   • Suppression of endothelial Kir current by atherogenic lipoprotein profiles in vitro and in vivo (Levitan)
   • The Dynamics and Mechanics of Endothelial Cell Spreading (Hammer)
   • Cholesterol regulation of membrane stiffness in endothelial cells (Levitan)
   • Differential stiffness sensing by cells (Janmey)
   • Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion (Weaver/Janmey)
   • Tensional homeostasis and cell phenotype (Weaver/Hammer)
   • Patterning, prestress, and peeling dynamics of cells (Discher)
   • Mechanics of the cell nucleus (Discher)
   • Strain stiffening in biopolymer gels (Janmey)
   • Force transduction mechanisms in cells (Janmey w/collaborator Weitz at Harvard)
   • Biophysical basis of angiogenesis directed through cell-substrate forces (Hammer)

2. at the in vivo and ex-vivo level of tissue investigations of blood vessels, heart valves, and site-specific therapy:
   • A new technique for the isolation of high quality RNA from small regions of the endothelium in situ and its application to heart valves (Davies)
   • Spatial heterogeneity of endothelial phenotypes and side-specific vulnerability to calcification in aortic valves (Davies)
   • The identification of molecular cues regulating ex vivo vein remodeling (Gooch)
   • The potential of endothelial cells to transdifferentiate and calcifiy (Levy)
   • The role of MMP9 in valvular calcification as assessed in RNAi and transgenic mouse investigations (Levy)
   • Transgene expression level and inherent differences in target gene activation that determine the rate and fate of cell differentiation in vitro (Gooch)
   • Valve disease-related gene identification (Levy)
   • Nitric oxide activation of the endothelial glucocorticoid receptor (Diamond)
   • Viral vector gene delivery for vascular disease (Levy/Diamond)

ISSUES

No significant negative issues. There are no barriers to group interactions of the participants; the institute structure helps facilitate this. All groups are located close to each other. BRP work is presented at institute and departmental seminars and chalk talks.

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PROJECT TITLE: Image-Guided Intracardiac Beating Heart Surgery

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT
 Modem cardiac surgical practice involves the routine use of cardiopulmonary bypass (CPB) for performing both coronary artery bypass graft (CABG) procedures on the heart surface as well as procedures inside the heart, classified broadly as intracardiac surgery. However, recent studies indicate that CPB carries important risks that can lead to reduced neuropsychiatric function and stroke in adults, and neurodevelopmental deficits with impaired fine motor skills in children. Other adverse effects of CPB include activation of inflammatory mediators and the complement cascade, showers of particulate emboli with aortic manipulation and crossclamp release, and air embolus. To avoid these risks of CPB, several investigators have begun to evaluate the results of CABG procedures performed without CPB. Early results of these "beating heart" procedures indicate equivalent patency rates, comparable mortality rates, and significant savings. Development of techniques for intracardiac beating heart surgery, however, must overcome the unique challenge of the inability to image the anatomic features of the heart with sufficient detail and time resolution to permit instrument navigation and precise tissue manipulation. Real time 3D echo has the potential for overcoming these issues thereby enabling intracardiac beating heart surgery. The overall aim of this proposal is to adapt real time 3-D ultrasound imaging specifically for image-guided interventions and integrate this technology with safety measures through instrument tracking, tactile sensing, and acoustic tissue analysis to permit safe and accurate intracardiac beating heart surgery. The complexity of this problem is well suited to a BRP approach. The PI has assembled a multidisciplinary team and established a unique partnership among industry-based engineers (Philips Medical Systems), university-based engineers (Harvard University; Boston University), and clinical investigators (Children's Hospital; Brigham and Women's Hospital). Together, we will approach this problem by addressing the following specific aims: AIM I: Modify real-time 3-D ultrasound to optimize image presentation for guiding intracardiac surgical procedures in a beating heart. AIM II: Adapt high-resolution electromagnetic tracking equipment for precise intracardiac navigation and modify surgical instruments to limit interference with ultrasound imaging during beating heart surgical procedures. AIM III: Develop instruments to provide both tactile sensing and acoustic tissue analysis for increased procedure safety. AIM IV: Integrate real time 3-D ultrasound imaging and tracking equipment with computer-enhanced instrument control for improved task performance and safety during image-guided surgery.

STATUS OF RESEARCH AND PARTNERSHIP
 AIM I - Modify real-time 3-D ultrasound to optimize image presentation for guiding intracardiac surgical procedures in a beating heart.
 We have modified the existing beam-forming software to provide a wider imaging frustrum to assist in navigation by providing a larger field of view. We have also developed an "n-way" parallel processing volume rendering capability. Another limitation in image guided surgery has been lack of depth cues in

the volume rendered images. We have incorporated this into the BRP Live 3D software for the streaming renderer. We can now run a 2 way pipeline for high definition stereo rendering. In in-vitro experiments, we coupled this to a high definition stereo display that tracks pupil movement of the surgeon. Stereoscopic display of volume rendered US images will likely enhance depth perception and ease of navigation by the surgeon.

AIM II: Adapt high-resolution electromagnetic tracking equipment for precise intracardiac navigation and modify surgical instruments to limit interference with ultrasound imaging during beating heart surgical procedures.

To provide a complementary method of instrument tracking within the surgical field, we developed a system to calibrate surgical instrument tracking . The setup consists of an accurate 5 axis electromagnetic positioning system, that accurately measures the instrument position with mechanical linear and angular positioning devices. This setup is to be used to calibrate and validate any instrument tracking algorithm or device we develop in the controlled laboratory setting before being used in surgical trials. To enhance instrument visualization we have developed an image processing with segmentation scheme. Statistical distribution of blood, instrument and tissue in intracardiac procedures was first determined and used to correct for voxel assignment within a static image. Neighboring voxel information and instrument shape are used in an iterative process to label voxels.

AIM III: Develop instruments to provide both tactile sensing and acoustic tissue analysis for increased procedure safety.

Force feedback capabilities integrated into surgical tools will offer the surgeon enhanced capabilities for accurately positioning surgical tools during beating heart ASD closure and MVA. In order to do precise assessment of instrument localization onto tissue touch-down sites, we developed an ultrasound sensor and hardware & software to drive this sensor. This has sub-millimeter resolution unlike the LIVE 3D software.

AIM IV: Integrate real time 3-D ultrasound imaging and tracking equipment with computer-enhanced instrument control for improved task performance and safety during image-guided surgery.

As a primary deliverable from Philips required for all parallel processing of ultrasound data and for remote control of image orientation, ultrasound external streaming capability was required. The reason for this capability was to allow research in Live 3D image analysis and tracking from the Philips Live 3D SONOS 7500 platform. The Philips group has designed and implemented hardware and software modifications to the SONOS system to allow streaming onto a desktop or laptop PC while surgery is taking place. This allows all BRP members to develop computer vision and robotic tracking developments for the life of the grant (and beyond.) This engineering development will facilitate integration of instrument tracking data, m-mode acoustic sensing data, and stereoscopic display of 3D image data. Building upon the streaming capability, we have generated a software structure to allow remote control of the LIVE 3D software. For example, if a virtual reality, tracking, or robot control needs to brighten or steer slices from a volume, we allow changes in render states to be transmitted and are building slice extraction capability as well.

ISSUES

Early on in the first year, subcontracting and agreements between the various institutions relating to intellectual property significantly delayed progress. These issues have now been resolved. No other issues have arisen during the past year.

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PROJECT TITLE: An Implantable Device To Predict and Prevent Seizures

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GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke

ABSTRACT

As many as 40% of individuals with epilepsy do not have their seizures controlled by current medical or surgical treatment. The need for new treatments is clear. We have assembled an ensemble of established investigators from the University of Pennsylvania, Georgia Institute of Technology, Children's Hospital of Philadelphia in a 5-10 year effort to create a novel therapy for refractory epilepsy: an implantable device capable of predicting epileptic seizures prior to electrical onset and triggering intervention to prevent their clinical expression. This complex task requires the focused efforts of a core of bioengineers from Penn and GIT in concert with experts in the fields of computer science, electrical engineering, clinical adult and pediatric epilepsy, neurophysiology, neuropharmacology and molecular and cellular neuroscience. This research partnership has three major thrusts: (1) Seizure Prediction: Developing and refining algorithms capable of predicting seizures hours to minutes prior to electrical and clinical onset. These algorithms are based upon signals obtained from implanted biosensors in adults, children and animal models of human epilepsy, (2) Mechanisms of Ictogenesis: Unraveling the neurophysiologic, neuronal network, cellular and molecular, mechanisms underlying the preictal (preseizure) changes identified by these algorithms through in-vitro and in-vivo investigations in the laboratory and clinical settings. Experimental observations will be incorporated into computer simulations of these mechanisms to facilitate development of better prediction and intervention strategies, (3) Therapeutics: Developing interventions aimed at specific points in the "ictogenic" process based on electrical brain stimulation to disrupt the cascade of events leading to seizures while preserving normal brain function.

STATUS OF RESEARCH AND PARTNERSHIP

Seizure Prediction, Seizure Precursors and Algorithm Development. In the fourth year of this project we continued to make progress in four areas: (1) Algorithm development: refining methods for detecting seizure precursors and identifying periods of increased probability of seizure onset, (2) application of engineering principles to brain stimulation to pre-empt and abort seizures, and (3) using these tools to gain more insight into mechanisms underlying seizure generation. Finally, (4) progress is being made in translating some of the seizure prediction results into a first generation implantable clinical device for treating epilepsy. NeuroPace, Inc., is currently enrolling patients in a prospective, blinded, controlled clinical trial of a responsive, implanted brain stimulation device to predict/detect seizures and stimulate to prevent clinical symptoms. Components of the seizure prediction technology are based upon algorithms licensed from The University of Pennsylvania and the Georgia Institute of Technology. Refining these algorithms is one component of the goals of this grant.

Mechanisms underlying ictogenesis. As part of the strategy to detect specific cellular and network behaviors underlying ictogenesis, we have continued to concentrate our efforts during in four fronts: (1) Developing multisite recordings of local field potentials and units from neocortex, thalamus and hippocampus, in order to detect the evolution of events during ictogenesis, simultaneously among these three structures, (2) Developing recordings of units in chronically implanted animals using tetrodes, (3) Determining particular susceptibility to seizures among different genetic mouse models, (4) Extending the recordings to awake, unanesthetized rats.

Circuit and cellular biophysical mechanisms during generation of the preictal cascade. We have spent the past year examining how seizures initiate in the limbic system, with a particular focus on factors regulating seizure entry into the hippocampus. To conduct this circuit level analysis with sufficient spatial and temporal resolution, we have used voltage sensitive dye imaging (VSD) techniques, and a very fast 80X80 CCD camera which allows us to sample activity at frequencies of 1-5 kHz and simultaneous patch clamp and field potential recordings. Using these VSD recordings, we have determined that the dentate gyrus acts as a filter, determined primarily by feedforward and feedback GABAergic inhibition, regulating entry of information and seizure activity into the hippocampus. This filter behavior may also be unusually dependent on extrasynaptic GABA receptors. We have also determined that seizure activity can spread from CA1 back into CA3 by the temporoammonic pathway, probably due to a loss of inhibitory interneurons and sprouting in this hippocampal region.

Application of Gene Transcription Assays as a Predictive Strategy in Ictogenesis. In order to define gene expression changes in a variety of human epilepsy syndromes and in several animal epilepsy models, we have optimized the methodology of mRNA amplification and cDNA array analysis in control rats from the animal core to establish critical baseline levels of expression. We have identified changes in gene expression in epileptic cortex around focal cortical lesions.

Network Mechanisms of Seizure Progression: Computational Modeling and Simulations. Models of the dentate-CA3 axis have been developed that focus on the potential for the dentate to drive CA3 to anomalous activity (seizures) and now model the potential importance of extrasynaptic GABA receptors in controlling excitability of the dentate gyrus.

Seizure suppression by brain stimulation in animals with seizures. We are now recording chronically from bilateral hippocampi in rats made epileptic with pilocarpine-induced status epilepticus and are identifying patterns of EEG activity that are correlated with impending seizures. In addition, we have begun preliminary open loop stimulation of unilateral hippocampus to try to alter the seizure pattern.

ISSUES

Developing animal models of either acute or chronic seizures that appropriately mimic the human condition and which can be utilized for the brain stimulation suppression experiments has proven to be a larger challenge than previously considered. Multiple animal models exist, but it remains to be determined how well any of them accurately reflect the human condition. Despite these caveats, the seizure prediction activities are proceeding rapidly in both humans and the animal models.

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PROJECT TITLE: High-Throughput Solid-Phase Combinatorial Biocatalysis

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GRANTING NIH INSTITUTE/CENTER: National Institute of General Medical Sciences and National Institute of Biomedical Imaging and Bioengineering

ABSTRACT

Rapid developments in genomics, proteomics, and combinatorial chemistry have reshaped the field of drug discovery, providing new drug targets for selective screens and new compounds to be tested in those screens. While combinatorial methods have given rise to large libraries of compounds, typically these compounds result in improved lead candidates that must undergo further transformations by conventional medicinal chemistry to yield new drug candidates. Bioengineering, in the context of high-throughput combinatorial methodologies, has not impacted lead optimization nearly as much as it has lead discovery, mainly because of the highly selective, intricate chemistries often required to optimize lead compounds and the lack of a suitably broad high-throughput platform. Combinatorial biocatalysis can help overcome these obstacles by exploiting the exquisite selectivity and unique reactivity of enzymes and microbial biocatalysts; however, to date this technology is limited by the relatively low throughput of solution-phase reactions. We are focusing our Partnership on expanding the scope of combinatorial biocatalysis to include reactions on, and the generation of libraries from, lead molecules attached to solid and soluble polymer supports. In the process, we will develop a high-throughput, biocatalytic technology for drug discovery. A series of lead molecules will be used in this work, ranging from enzyme substrates that are attached onto solid and soluble polymer supports to complex compounds (the flavonoid bergenin and the current HIV-1 protease inhibitor indinavir). Successful completion of this research program will result in a powerful methodology that can be used by biomedical investigators in the search for new, more potent small molecule therapeutics.

STATUS OF RESEARCH AND PARTNERSHIP

In our first year of support, we have expanded the scope of our combinatorial biocatalysis technology to include solid-phase reactions for the generation of lead compound libraries on solid and soluble polymer supports, as well on microarrays (Dordick and Clark). Several biocatalytic synthetic strategies have been performed on the solid phase. Specifically, enzyme-catalyzed C-C bond formation has been performed on solid-supported phenols, thereby expanding the repertoire of enzymatic catalysis on resin-bound substrates. Soybean peroxidase (SBP)-catalyzed the coupling of the NADPH oxidase inhibitor apocynin (acetovanillone) gave dimers, trimers, and oligomers with structures similar to what is obtained in solution-phase reactions. In addition to SBP, CPO-catalyzed chlorination/bromination of resin-bound p-hydroxyphenylacetic acid was successfully performed. SBP catalysis has also been performed on microarrays at the 10 nL spatially addressable reaction scale (525 spots/slide). A seed phenol was attached to the glass surface chemically, which acts as a substrate for SBP catalysis in the presence of a suitable phenol and H_2O_2. The presence of oligophenols on the glass slide was confirmed by incubating the treated slides with an amine-reactive fluorescent dye, which binds to the free primary amine of tyramine and can be detected using a fluorescence-based scanner. Different H_2O_2 concentrations were

spotted, which resulted in different amounts of tyramine attached to the slide. Multiple enzymes and substrates are now being employed in a combinatorial or iterative fashion to generate microarrays of small molecules for biological screening in high-throughput activity.

To support the combinatorial functionalization of lead molecules, three programs are underway to design more efficient enzymes with tunable selectivity. Clark and Dordick have developed a novel enzyme solubilization technique for use for lead optimization reactions in nonaqueous media involving organic solvent-soluble subtilisin Carlsberg, C. antarctica lipase B, and SBP. Surprisingly, the activity was high in polar organic solvents. Characterization of the solubilized enzymes via light scattering showed them to be a structurally unique complexes consisting of large molecular aggregates of ~60 protein molecules within each protein-surfactant ion-paired complex for subtilisin and ~100 molecules/aggregate for lipase. It is believed that it is this aggregate formation upon direct solubilization this is responsible for the dramatic increase of solubilized enzyme activity.

Klibanov has aimed to understand to what extent enzymatic activity correlates to active site amino acid sequence. A simple natural algorithm was designed that produces native active-site sequences of enzymes, with the Streptomyces R61 DD-peptidase chosen as a model system. In the DD-peptidase active site, six of the seven residues involved in contacts with the substrate - F120, N161, W233, R285, T299, and S326 – were correctly predicted. The generality of the algorithm to predict the active site amino acid sequence of enzymes was further tested by applying it to thymidylate synthase and -galactosidase. As a result, the algorithm correctly predicted 78% of residues from both enzymes, with 83% similar to native (90% correct, with 95% similar, excluding residues with high variability in multiple sequence alignment).

Chemical modification of enzymes is being pursued by Davison to increase the diversity of enzyme reactions available on solid supported substrates. Cytochrome c and CPO were modified using alkyl aldehydes and screened for enantioselective sulfoxidation and epoxidation activity. Preliminary results show that both reactivity and enantioselectivity are governed by the length of a simple alkyl chain bound to cytochrome c. More significant changes in activity and selectivity are now being achieved by combining chemical functionalization with structure-based design.

ISSUES

A website for the project to allow data transfer and better interaction of all project members was developed and is available at http://htcb.ornl.gov. All subcontracts have been running smoothly.

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PROJECT TITLE: Rapid Flow Evaluation by Magnetic Resonance Imaging

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT

Velocity encoded cine (VEC) imaging performed using magnetic resonance imaging (MRI) has great clinical potential for diagnosis of cardiovascular diseases. The non-invasive nature of MRI tomographic imaging, its uniform sensitivity to velocity in all directions and its intrinsic 3D nature make it a natural choice for clinical application. Of particular interest is the potential use that can be made of quantitative blood velocity imaging in the assessment of the complex flow fields associated with aortic valvular diseases. Currently, aortic valve diseases are primarily assessed using echocardiography which is widely available, but nevertheless has several important limitations in characterizing flow fields, including views are restricted by the availability of appropriate acoustic windows, results are operator dependant, velocity is detected in only one direction relative to the probe and that primarily 2D views are used to characterize a 3D flow field.

While MR VEC imaging has the potential to provide more comprehensive flow field data than does echocardiography, clinical application of MR VEC imaging has been hampered by its relatively long acquisition times. The powerful gradient systems now available on MRI scanners allow high quality cardiac cine scans to be acquired in comfortable breath-hold times. However, the scan time required for VEC imaging with velocities resolved in 3D is still prohibitively long for most clinical applications. The goal of this proposal is to implement a rapid MRI approach that has potential to accomplish VEC imaging in a conventional breath-hold time. Development includes MR scanner sequences modification, determining its limits of applicability using computer modeling of flow fields and testing using flow models. In parallel with implementation and validation of the acquisition sequence, processing tools will be developed to analyze the time resolved 3D flow field data sets. Following the development stage, clinical application will be made to patients with aortic valvular diseases.

STATUS OF RESEARCH AND PARTNERSHIP

We have successfully implemented the basic Block Regional Interpolation Scheme for K-Space (BRISK) acquisition that allows VEC data to be acquired in as little as 20% of the conventional scan time for segmented k-space approaches. We have conducted computational fluid dynamic (CFD) investigations into the complex flow patterns in curved tubes and showed that BRISK and variations on BRISK can accurately represent major flow characteristics quantitatively. CFD calculations have shown that adequacy of temporal MRI flow data is the dominant factor affecting accuracy when studying pulsatile flow. BRISK allows adequate temporal resolution to be achieved in representing pulsatile flow. Investigations have been conducted into issues associated with slice thickness and orientation for the calculation of control volumes for convergent flow patterns associated with restrictive cardiac values. We have shown that for MRI data with adequate temporal resolution, accurate representation of flow is dominated by slice orientation, which should be arranged such that the slice thickness dimension is oriented along the direction with the lowest flow gradient. As part of the project, we have sought to

optimize the implementation of BRISK. Following CFD simulations, we deconstructed the acquisition into a BRISK component and a conventional k-space segmentation component. These simulations indicated that, for a given scan time, better accuracy could be obtained by increasing the BRISK component while decreasing the segmentation component. This led us to develop a variant termed FRISK (Fragmented Regional Interpolation Scheme for K-Space) in which the sections of k-space that are sampled are not treaded as discrete blocks but are explicitly treated as temporally distributed data. The temporal interpolation processing required to construct complete k-space maps specifically accommodates the exact temporal order of the data in FRISK. The FRISK data sets have lower artifact than conventional BRISK.

An issue recently investigated is the direct visualization of jet flow. Jet flow is problematic for MRI due to the high acceleration terms involved. While increasing the temporal resolution is necessary for accurate jet visualization, it is not sufficient. An important issue is the degree of temporal misregistration that exists for the flow reference and flow encoded scans, and typically, this cannot be eliminated by conventional scans, even if high temporal resolution data is acquired. Temporal misregistration can result in dramatic overestimation or underestimation of jet flow (exceeding 100% error). The temporal interpolation feature of our rapid imaging approach can be applied to our own data as well as conventionally acquired data to achieve temporal registration at the post processing stage. We show by simulation and direct acquisition, that accurate representation of jet flow is possible using the processes developed as part of this BRP.

ISSUES

The partnership is working well. We have found that each investigative arm enhances understanding in the other disciplines involved. This has led to a greater depth to the research. The initial emphasis of the research was to image the convergent flowfield, since direct visualization of jet flow was considered beyond the capabilities of MRI. However, the processing developed to improve our rapid BRISK scan was extended to represent jet flow accurately and directly. Another aspect of the project that was enhanced by the cross-disciplinary nature of the investigation was the appreciation for features other than scan speed that affect accuracy of flow data. In the literature, slice orientation relative to flow is usually discussed in terms of scan efficiency. Our simulations in this area showed that accuracy and efficiency varied dramatically depending on slice orientation. Also, for any given slice orientation series, computation of the associated control volume surface area should take in to account the exact nature of the intersecting slices of an acquisition series. Further, the representation of the typically much lower in-plane flow features in complex flowfields has traditionally been problematic for MRI VEC data. We have analysis to suggest that this is not merely a problem associated with dynamic range of the data. Investigations are underway to analyze this data to make BRISK and its variants more sensitive to these features. In summary, we are very encouraged by the partnership and believe that its very structure has contributed to this research project.

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PROJECT TITLE: Bioimaging and Intervention in Neocortical Epilepsy

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering and National Institute of Neurological Disorders and Stroke

ABSTRACT

Magnetic resonance functional and spectroscopic imaging (fMRI, MRS) of the brain provide tremendous opportunities in the study and treatment of epilepsy. In neocortical epilepsy, where the epileptogenic region is highly variable in size, structure and location, deeper insight into the biochemical and functional characteristics of the region and surrounding tissue may provide critical data to assist the neurosurgeon and neurologist in localization and treatment. To fully utilize the multiple forms of available information (MR and EEG), these data must be transformed into a common space and integrated into the intraoperative environment. The work being performed on this grant will develop high resolution MRS and fMRI at 4T and advanced analysis and integration methods to better define the epileptogenic tissue and surrounding regions, and enhance our understanding of the biochemical mechanisms underlying the dysfunction in neocortical epilepsy. We will validate these measurements against the gold standard of intracranial electrical recording. These goals will be achieved in this bioengineering research partnership (BRP) by bringing together six partners from 3 academic institutions (Yale (lead institution), Albert Einstein and the University of Minnesota) and industrial partner (BrainLAB, AG) to carry out four integrated programs of scientific investigation and bioengineering development in the area of bioimaging and intervention: 1) development of high resolution fMRI and MRS at 4T for the study of epilepsy; 2) investigation with MRS of the relationship between neuronal damage or loss through the measurement of N-acetyl aspartate (NAA), alterations in neurotransmitter metabolism through the measurement of gamma amino butyric acid (GABA) and glutamate, and abnormalities in electrical activity in the epileptogenic region and surrounding tissue; 3) investigation of the relationship between fMRI activation amplitude and the cognitive task, underlying cortical structure, cortical metabolic state, and physiology, and the impact of epilepsy on these factors; 4) development of integration methodologies for fusing multimodal structural and functional (image- and electrode-derived) information for the study and treatment of epilepsy.

STATUS OF RESEARCH AND PARTNERSHIP

Our efforts continue to proceed as planned, with progress related to each of the original aims noted above as follows:

1. *Coil Development for High Resolution MRI/MRS:* In the past year, we have demonstrated the significant advantage of our actively detunable, phased array TEM coil for depths under approximately 5 cm. In order to achieve substantial SNR increases in comparison to conventional volume coils from the central region (7-8cm depth) of a human head, we have designed a counter

rotating surface coil (CRC) consisting of two parallel rings carrying opposite currents. This permits an intrinsic isolation of the surface coil from the transmit coil, which enables simultaneous reception by volume and surface coils. We have achieved up to 35% SNR improvement near the head and phantom centers. We have demonstrated spectra acquired simultaneously from the CRC array and the volume coil from locations near the posterior surface and the middle of the brain.

2. *MRS - Biochemical Imaging of Epilepsy:* We have been measuring NAA and glutamate as markers of epilepsy. The primary limitation to the measurement of glutamate, even at 4T, is the inability to obtain adequate homogeneity for resolution of glutamate from overlapping resonances such as glutamine. To address this, we have developed a novel B0 mapping method using multiple evolution times with a novel unwrapping scheme in combination with a user defined ROI selection tool. In data acquired from 10 subjects, the mean difference between the achieved homogeneity and the predicted best homogeneity was 1.22 ± 0.62Hz after a single iteration and 0.67 ± 0.37 Hz after two iterations. We have also acquired NAA images with 125µl voxels and a SNR of 10-12.5:1 from about 20 human subjects (both normals and epilepsy patients). Using the image analysis strategies described below we have performed initial experiments to relate MRS imaging measurements of NAA to electrophysiologic data revealing a spatial correlation linking abnormal electrical spiking (higher spike counts) and abnormal NAA levels (lower NAA/creatine ratios) in epileptogenic regions.

3. *fMRI:* We have incorporated RF field map data and receiver reception sensitivity data into our routine fMRI analysis. These data are helpful for later segmentation of the brain using high resolution data, and then partial volume correcting the fMRI data to obtain a better regional assessment of activation and the center-of-mass of activated regions. Such adjustments to the center-of-mass determination are important for comparison studies such as those between MRS and/or data from subcortical electrode stimulation studies. Related to this work we have assessed the inter- versus intra-subject variance at high field (3T/4T) in an effort to determine the optimum resolution for single subject and multi-subject studies. The results indicate unique parameters must be used for individual subjects in the neurosurgical planning environment.

4. *Integrated Image Analysis:* We continue to make progress in the development of integration methods and in our image analysis platform. Work on brain shift compensation has moved away from employing blood vessels as features (difficult to find from MRA near the surface) and we are now investigating the use of sulcal features within our framework. Testing on phantoms and initial human image data is being carried out. For implanted electrode localization, we have further developed our methods to localize them as defined from their 3D coordinates in registered CT and MR images. This capability has become part of the clinical routine for these patients (22 patients have been processed) and facilitates the comparison of electrode recordings with our pre-operative MR-derived measurements. The BrainLAB and the Yale Image Analysis partners completed initial testing of a research interface that was jointly developed for the VectorVision Image Guided Surgery system that facilitates the safe incorporation of new algorithms in the operating room.

ISSUES

All partners have been communicating effectively. Discussions are underway regarding directions for competitive renewal.

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PROJECT TITLE: In Vivo EPR Bioengineering Research Partnership

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ABSTRACT
Electron Paramagnetic Resonance (EPR) spectroscopy detects unpaired electrons. It is being developed as a tool for monitoring local oxygen concentrations in vivo via the impact of the paramagnetic oxygen on probes with narrow oxygen-dependent lineshapes. To study radicals deep in tissues it is necessary to perform EPR at radiofrequencies where the inherent sensitivity is lower than at the microwave frequencies that are typically used for ex vivo spectroscopy. Much of EPR spectroscopy is performed with magnetic field scans that are slow relative to the linewidth (CW EPR) or by applying pulses of incident radiation (pulsed EPR). There is an intermediate case in which the magnetic field is scanned rapidly through the signal, but it has not been used in EPR because of the need for specialized hardware and the need to process the signal to remove distortions introduced by the rapid scan. However, this approach is expected to be advantageous when dealing with rapidly changing signals and for optimizing scan rate relative to physiological motions. The specific tasks include the design, construction, and testing of an air-core magnet system for scanning the magnetic field rapidly. The noise characteristics of the spectrometer and of living samples will be analyzed to optimize scan rates. Software will be written to deconvolute the undistorted spectrum from the experimental lineshape. The deconvoluted spectra will be used to reconstruct images that include both spectral and spatial dimensions.

STATUS OF RESEARCH AND PARTNERSHIP
In the past year we have made substantial progress in both instrumentation and data analysis. Initial experiments were performed with sinusoidal field sweeps because these are available in commercial instrumentation, which facilitated exploratory experiments. However, in the sinusoidal sweeps the scan rate changes continuously across the sweep, which complicates recovery of the slow-scan lineshape. We have designed and constructed a system that generates triangular sweeps with sweep frequencies between 1 and 10 kHz, sweep widths of 1 to 10 G, and good linearity over approximately 90% of the sweep range. In these sweeps the scan rate is constant, which permits us to use Fourier deconvolution to restore the slow scan lineshape with high spectral fidelity. Accurate lineshape information is key to our goal of monitoring local oxygen concentration based on broadening of the EPR signals. Design of a second generation triangle scan driver that will provide wider sweeps is underway.

Direct-detect rapid-scan EPR spectra at 9.8 GHz were obtained using a Bruker E580 spectrometer. Spectra of an aqueous solution of Nycomed triarylmethyl (trityl-CD3) radical ($T1 = 11.5$ µs) were recorded at scan rates between 3.4 kG/s and 750 kG/s. Signals for LiPc were obtained with a split ring resonator, a rectangular resonator, and a dielectric resonator. At faster scan rates the small bandwidth of the high-Q dielectric resonator filters out high frequency components of the rapid-scan signals. Field inhomogeneities induced by the rapidly changing magnetic field increase with scan rate and are greater with the dielectric and split ring resonators than with the rectangular resonator. The ~3 mm-long extended

trityl sample shows larger effects of magnetic field inhomogeneities than the small LiPc crystals. These experiments help us to understand instrumental characteristics that will be crucial to system optimization.

Direct-detected rapid-scan EPR signals at 250 MHz have been recorded using triangular field scan rates between 1.7 kG/s and 150 kG/s for deoxygenated samples of lithium phthalocyanine (LiPc) and Nycomed trityl-CD3, which are very sensitive oximetric probes. These scan rates are rapid relative to the reciprocals of the electron spin relaxation times and cause characteristic oscillations in the signals. Fourier deconvolution with an analytical function permitted recovery of lineshapes that are in good agreement with experimental slow-scan spectra. Unlike slow-scan EPR, direct detection rapid-scan EPR does not use phase sensitive detection and records the absorption signal directly instead of the first derivative of the absorption signal. In EPR imaging magnetic field gradients are used to encode spatial information. A major advantage of our rapid scan approach is that it provides the absorption signal directly, without need for signal integration. The signal-to-noise in the absorption signal decreases linearly with gradient, whereas the signal-to-noise for a traditional first-derivative EPR signal decreases quadratically with gradient.

Images of phantoms constructed from samples of LiPc and trityl-CD3 were reconstructed by filtered back-projection and by maximum entropy methods from data sets with a missing angle. The lineshapes in spectral slices from the image are in good agreement with slow-scan spectra and the spacing between sample tubes matches well with the known sample geometry. We are currently comparing the two methods of imaging reconstruction.

The collaboration with Bruker has provided hardware at reduced cost and invaluable information concerning their hardware and software. It is already providing input to their design considerations. Bruker is modifying their data acquisition software to incorporate an option for rapid scan. We have provided feed-back on a preliminary version and a working version is expected to be available in the near future.

ISSUES

We are pleased with this third year of this BRP and the useful collaboration between the University of Denver and Bruker BioSpin.

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PROJECT TITLE: Robotically Generated Locomotion in Rodents

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT

The adult mammalian lumbar spinal cord can learn to step in the absence of descending input from the brain. The ability of the spinal cord to learn is an extremely important finding for tens of thousands of spinal cord injured patients, as it could mean the difference between being confined to a wheelchair or being able to stand and take some steps. Understanding how to teach the spinal cord to step through effective rehabilitative training has immediate clinical application in itself and can also play a crucial role in enhancing the efficacy of other potential therapeutic interventions for spinal cord injuries. One method of rehabilitative training, i.e. body weight supported locomotion on a treadmill, has been successful in enhancing locomotor recovery in spinal cord injured animals. There is growing evidence that this form of training can also be used to improve walking in humans that have suffered a stroke or spinal cord injury. The success of training, however, depends on the generation of appropriate patterns of sensory information during weight bearing stepping. We have developed a robotic system to train the hindlimbs of spinally transected rodents to step on a treadmill. The robotic system provides precise control of forces acting on the hindlimbs during stepping and also provides on-line measurement of step cycle trajectory characteristics so that locomotor performance can be quantified quickly and objectively. We hypothesize that the recovery of hindlimb stepping in spinally transected rats will be enhanced by robotic-controlled locomotor training.

a. *Purpose.* The overall aim of the project is to examine the effects of robotic training on hindlimb stepping in spinally transected (ST) rats.
b. *Methods.* In the first two years of the project, we found that the robotic-enhanced loading on the hindlimbs altered the kinematic characteristics of the step cycle in ST rats and that use of the robotic device to fully assist stepping was not effective in enhancing stepping in ST rats. In the third year, we tested the hypothesis that the ability of the lumbar spinal neurons to generate stepping was dependent on the amount of robotic training that was imposed. The robotic device was used to control the amount of steps that ST rats performed during each training session. If our hypothesis was correct, then the rats that received the greatest amount of hindlimb training would recover the best stepping function. In the third year, we also started development on a new robotic training device (e.g. the "slide") that we will use to study the effects of robotic control over hindlimb coordination during training (year 4). Unlike a conventional treadmill in which the two hindlimbs move on one treadmill belt, the slide device consists of two moving platforms, one for each hindlimb. This allows control over the phasing of movements in the hindlimbs and we will use the slide device to test the hypothesis that alternating movements during training provide optimum sensory cues to the spinal cord for stepping.

c. *Studies and Results*
   1. Recovery of stepping in ST rats is dependent on the amount of robotic training
      Two groups of ST rats were trained, one group performed 1000 steps/training session (n=13) and the other group performed 100 steps/training session (n=12). Robotic arms were attached to the ankle of the rats during treadmill training and software was developed to count the number of steps performed by the hindlimb based on the horizontal movement of the robotic arms. Only step cycles that were greater than 1 cm in length were counted thereby eliminating small stepping movements. After 4 weeks of training, the 1000 step group recovered better stepping ability than the 100 step group. For example, the 1000 step group performed more medium-sized and large-sized steps than the 100 step group. In addition, the maximum amount of weight bearing during stepping decreased in the 100 step group but not in the 1000 step group.
   2. Development of new robotic device for controlling hindlimb coordination during stepping
      The slide uses two direct drive, electric linear motors (LinMot PS01-23x80) to move two platforms that contact the hindpaws. The advantage of the slide over a conventional treadmill is that it allows independent control of the position of the two hindlimbs. For example, one hindlimb can be brought backward (i.e. simulating stance), while maintaining the other hindlimb in a static position. Thus, the effect of contralateral hindlimb position on the probability of initiating swing in the ipsilateral hindlimb can be studied.
d. *Conclusion.* These findings suggest that robotic training improves the ability of the lumbar spinal cord to generate hindlimb stepping. Furthermore, other factors related to the experience of robotic training, i.e. getting used to the robotic arms, handling, etc., do not play significant roles in stepping recovery since ST rats that were exposed to the robots but experienced only minimal stepping (100 steps/day) recover poorer stepping ability than the ST rats that received a greater amount of training (1000 steps/day). These findings have important implications for gait training therapies that are currently used for spinal cord injured humans. Based on the results of the third year experiments, we will proceed as originally proposed and in the fourth year, we will test the effects of robotic controlled hindlimb coordination using the newly-developed slide device.

STATUS OF RESEARCH AND PARTNERSHIP
   In the second year of the project, we performed experiments that addressed the effectiveness of full versus partial mechanical assistance during treadmill training in spinally transected rats. We tested the hypothesis that the lumbar spinal cord adapts to the levels of assistance provided during treadmill training. For example, fully assisting the hindlimbs during the step cycle, and thus not allowing the spinal cord to generate independent stepping would result in poor walking recovery. Conversely, providing assistance only when needed would promote better locomotor recovery. A robotic device developed in Dr. Dave Reinkensmeyer's laboratory at UC Irvine was used to implement the hindlimb training in spinally transected rats. The device consists of two small, lightweight arms that attach to the rat's hindlimbs and a weight support apparatus that controls the amount of weight bearing on the hindlimbs. To develop a full assistance training algorithm, we recorded the movement of the robotic arms while an experienced trainer manually trained the hindlimbs of an ST rat to step on the treadmill. The resulting X-Y trajectories were recorded by the robotic arms and served as starting point for a "trainer-based" algorithm. We used the full assistance algorithm to train the hindlimbs of 13 ST rats daily for 5 days/week for 4 months. These experiments were performed in Dr. Ray de Leon's laboratory at Cal State LA. The training protocol consisted of 30 minutes/day of continuous robotic training in which the two hindlimbs were moved through thousands of step cycles using the "trainer-based" trajectory. After 4 months of full assistance training, there was no difference in the mean number of steps performed by the trained and non-trained rats, suggesting that training with full assistance did not provide the proper sensory cues necessary for learning to step. To determine if partial assistance training would improve stepping, the trained rats underwent an additional month of training in which assistance to the hindlimbs was administered by the trainers only when necessary (i.e. when the hindlimbs failed to move during stance or swing). After one month of partial-assistance training, more of the trained rats relative to the non-trained rats performed weight bearing steps during the robotic tests.

ISSUES
   The partnership among the three investigators, Dr. Edgerton, Dr. Reinkensmeyer and Dr. de Leon, has been productive and effective and there are no major issues to report.

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PROJECT TITLE: Spectroscopic Imaging and Diagnosis of Neoplasia

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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute

ABSTRACT

The goal of this Bioengineering Research Partnership is to develop a spectroscopic imaging methodology for diagnosing pre-invasive neoplasia (dysplasia) and monitoring its progression. The program is based on optical spectroscopic instrumentation and diagnostic algorithms which have been developed at the MIT G.R. Harrison Spectroscopy Laboratory. The instruments to be developed have two components, a system for wide-area imaging of neoplasia based on light scattering spectroscopy (LSS), and an optical fiber probe device (FastEEM instrument) for more detailed study of suspect regions based on tri-modal spectroscopy (TMS). The goal of the program is to develop and perfect the new technology and assess its application to the diagnosis, characterization, and therapy of neoplastic progression in human patients in real time. The detection and monitoring of neoplastic lesions in the oral cavity and the cervix will be used as model systems for establishing the potential of the technology. In addition, basic studies to further improve the technology and its ability to characterize pre-invasive neoplasia will be conducted. Six projects will be undertaken, each led by an experienced investigator: (1) Prototype instruments and diagnostic algorithms for clinical studies will be developed, maintained and perfected. Clinical studies will be conducted on patients with suspected lesions in the (2) oral cavity and (3) uterine cervix to evaluate and perfect the technology for diagnosing and monitoring dysplasia and predicting the patient's response to treatment. Two basic projects aimed at enhancing the diagnostic accuracy of the clinical instrumentation will be undertaken, one (4) to explore the use of quasi-multiple scattered light to enhance the sensitivity and provide depth resolution to LSS imaging, and a second (5) to develop novel spectroscopic end-points based on well-characterized molecular and cellular events associated with the progression and regression of disease. (6) Pathology support activities will include analysis of oral and cervical tissues for molecular markers, and analysis of histological sections of the same biopsy tissue by computer-assisted quantitative image analysis. An administrative core will coordinate the multidisciplinary activities of the program and insure information sharing and efficient communication. The partnership, composed of expert investigators at six institutions, will include experienced bioengineers with training in physics and mechanical/electrical engineering, pathologists experienced in cancer research, and hospital-based clinicians specializing in oral and cervical dysplasia.

STATUS OF RESEARCH AND PARTNERSHIP

Organizational Structure: This partnership brings together investigators from multiple academic institutions with expertise in optics, medicine and cellular biology. A plan to coordinate research activities of the group has been developed which provides for a tiered set of research meetings among various

groups, including semi-annual program meetings at which all project leaders and research staff review progress and discuss future directions. One of these program meetings also includes an external advisory committee with broad expertise in optics, spectroscopy, medicine and cell biology. The advisory committee critiques the directions and progress of the program annually.

Instrument Development and Integration: The specific aims of this project are to build and maintain FastEEM optical probe instruments and spectral imaging instruments for use in the clinical projects. We have already built and tested two optical probe instruments for use in the cervix and the oral cavity. The instruments are fully operational and are being used routinely. A clinical imaging instrument has also been built and is currently under further development in the laboratory. In addition, we continue to develop new software and improved diagnostic algorithms for real-time instrument control and automated data analysis for these instruments.

Development of Novel Spectroscopic Methodologies: The goals of this study are (1) to establish how LSS imaging of multilayered tissues is affected by multiple light scattering, and (2) to extend the capabilities of LSS to obtain complimentary information about tissue structure using multiple light scattering. So far, we have accomplished our goals for the first two years by developing a specialized light scattering instrument capable of collecting polarization dependent spectral, angular, and spatial information about light scattering by multilayered tissues. We have used the instrument to understand the mechanism of depth selectivity in polarization-gating, and continue our basic studies and modeling to obtain complimentary information about tissue structure using light scattering spectroscopy.

Development of Novel Spectroscopic Markers: These studies are designed to establish novel fluorescence and LSS markers based on molecular and cellular events that are known to be associated with squamous epithelial neoplasia. Specifically, we study molecular events associated with expression of the "high-risk" human papillomavirus (HPV-16) derived oncoproteins, which are commonly expressed in HPV-associated cervical and oral cancer. Over the past two years, we have set up fluorescence and LSS imaging instruments for microscopic studies of HPV oncoprotein expressing cell lines. In addition, we have developed experimental conditions for spectroscopic characterization of apoptotic events in cell culture. Our studies reveal and characterize spectral changes in cells after exposure to HPV oncoproteins and after induction of apoptosis. Lessons learned from these bench experiments will be incorporated into the clinical algorithms over time.

Clinical Testing and Validation: The primary goal of two separate clinical projects (cervical and oral neoplasia), as well as a quantitative/molecular pathology core, is to develop and perfect multi-modal spectroscopic algorithms for diagnosis and classification of pre-neoplastic lesions in the uterine cervix and the oral cavity. To date, the cervical project has collected data from 58 patients and the oral projects from 44 patients and 16 healthy volunteers. These data are at various stages of analysis and correlation with pathological diagnoses, tissue morphometric parameters and HPV status.

ISSUES

The rate of patient accrual for clinical studies has been slower than expected because of unexpected changes in key clinical personnel. This issue has been resolved by recruitment of new clinical collaborators.

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PROJECT TITLE: Integrated Ultrasonic Systems for Non-Invasive Therapy

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute and National Heart, Lung, and Blood Institute

ABSTRACT

Purpose. The ultimate objective of this 5-year Biomedical Research Partnership (BRP) is to develop a unified body of scientific knowledge and validated technology concepts that are needed to establish ultrasound as a practical non-invasive treatment modality and to inaugurate ultrasonic therapeutics as a new biomedical discipline.

Methods. The program develops integrated ultrasonic systems to position, induce, and monitor therapeutic lesions that modify various diseased tissues. Our multi-disciplinary research is designed to achieve a series of fundamental advances in the diverse areas involved in therapeutic ultrasound. It employs extensive theoretical modeling to elucidate physical ultrasound-tissue interactions that can be used to produce therapeutic changes in diseased tissues. The research validates model results for thermal and mechanical effects in a series of animal experiments. Validated results are used to design and implement advanced therapy systems incorporating ultrasonic arrays and real-time lesion monitoring. Systems are being tested and refined using animal experiments that investigate cancer and heart-disease therapy.

Results. Our results will be incorporated in a systems model of ultrasonic therapy that will permit comprehensive treatment planning and provide a basis for designing therapy systems.

Conclusions. The research is focused on establishing a comprehensive basis for ultrasonic treatments of cancer (primarily of the breast and prostate) and cardiac disease (primarily ventricular arrhythmia and myocardial insufficiency). These clinically significant diseases present challenging opportunities to test and refine our concepts, which have substantial implications for treating a broad array of problematic, life-threatening conditions.

STATUS OF RESEARCH AND PARTNERSHIP

This past year has seen several important advances arising from the close cooperation of the BRP partners.

One of the most challenging aspects of the project is ultrasonic monitoring of thermal HIFU lesions. The acoustic parameters of a thermal lesion are sufficiently similar to normal tissue so that standard B-mode ultrasound imaging fails to detect cold thermal lesions (although a signature of bubbles often occurs during and immediately following lesion formation). Riverside Research Institute (RRI) and Weill Medical College of Cornell University have modified RRI-developed spectrum analysis techniques (in which a power spectrum is obtained from the ultrasound backscattered data of a region of interest and a regression line is fit to the spectrum) to yield highly detailed views of lesions otherwise invisible to ultrasound.

The integrated therapeutic diagnostic ultrasound device that has been under development by Spectrasonics, Inc., has been completed and shipped to the Columbia University College of Physicians

and Surgeons, where it has been used to form lesions precisely where predicted in the cardiac ventricles in the chronic dog.

Linear and non-linear propagation models of high-intensity focused ultrasound (HIFU) lesion formation for cancer and cardiac therapies have been found to agree with each other and with in-vitro experiments over a clinically useful set of operating parameters. The linear model was generalized as a dynamic model to simulate HIFU lesion formation in the beating heart. Future work will involve validation with in vivo data.

Additionally, a United States Patent (6,846,290) has been obtained. It covers automated ultrasonic tracking to maintain a desired distance between a therapeutic ultrasound transducer and a targeted tissue structure. Such tracking can help to maintain focus during breathing and other patient motions as well as during the cardiac cycle.

ISSUES

Because of the death of the original Principal Investigator, Dr. Frederic L. Lizzi, Dr. Ernest J. Feleppa is leading the project as Principal Investigator in compliance with NIH procedures.

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PROJECT TITLE: Anti-Inflammatory Coatings for Biomaterials

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT

The prolonged inflammatory response to an implant is one of the primary causes for the failure to integrate into tissue. The two sources of inflammation common to almost all implants are the foreign body response and the relative movement of the implant with the surrounding tissue. Based on evidence in the literature and from our research team, the inflammatory response is mediated by the reactive oxygen species generated by macrophages, leukocytes, and the surrounding connective tissue. Based on our findings, it is evident that titanium dioxide and similar ceramics, even when present as surface coatings of polymeric biomaterials, have the ability to breakdown ROS that have been identified as mediators of the inflammatory response. The goal of this Program is to develop applications for our catalytic antioxidant ceramic technology in the biomaterials and medical device industry. This Program, led by LJBI, consists of five projects with eight academic and industrial partners. Project 1 will investigate the basic mechanisms of action of metal oxides in the catalytic breakdown of ROS. By understanding the fundamental reaction kinetics of the catalytic action of TiO2, catalysts of greater efficiency may be discovered. Project 2 will fabricate and characterize materials for the other four Projects, and partners with Lawrence Livermore National Labs, Drexel University, University of California, Uppsala University, and La Jolla Bioengineering Institute. Project 3 will test the in vivo inflammatory and foreign body response in two in vivo models; a standard rat model and the hamster window model. This project provides a core service to the other projects, but also investigates fundamental mechanisms of the inflammatory response to biomaterials. Project 4 will determine if the catalytic antioxidant ceramic technology is able to mitigate implant-tissue strain-induced inflammation. It will also investigate basic mechanisms of strain-induced inflammation. Project 5 is the interface with the medical device industry. Industrial partners have been chosen to develop applications in different biomaterials areas: Biosensor membranes for implantable glucose sensors (Advanced Tissue and Materials Inc), wound dressing material with anti-inflammatory properties (3M), and dental materials with improved osteointegration (Nobel Biocare). Our overall objective is to provide the proof-of-principle to our industrial partners, which will encourage them to participate in more specific product development.

STATUS OF RESEARCH AND PARTNERSHIP

Project 1: A breakthrough has been achieved in investigating the mechanisms of action of the titanium coatings. This has led to the development of a new class of biomaterials based on titanium. We have previously shown that TiO2 surfaces have anti-inflammatory properties. Here we investigate the effect the valence state of the titanium in a TiO2 structured crystal has on these properties. As this invention is being filed as a patent application, details will be presented at a later time.

Project 2: We have completed the micropatterning of biomaterials with titanium. The results demonstrate that a 23% coverage of microdots of the size 5 microns confers the same anti-oxidant

properties as full converage. We have submitted a manuscript for publication, and have discussed licensing of the technology with Medtronics.

Project 3: The important findings discussed in the Project 1 summary were confirmed in the in vitro studies of Project 3.

Project 4: The effect of implant-tissue strain was investigated in a rat-implant model where the magnetized implant is subjected to oscillating strain. We found that increased oscillatory strain induced a thicker foreign body capsule.

Project 5: We are collaborating with Philometron in the development of implantable biosensors. Philometron was recently awarded an SBIR grant on their titanium implantable biosensor where we are providing technical expertise.

We continue to collaborate with Applied Tissue and Materials Inc on the development of the micropatterned titanium biosensor membrane. We expect to submit a Phase 2 STTR to further develop this BRP-based technology.

Project 6: The development of near infrared imaging of the foreign body response is progressing with the acquisition of the necessary equipment for the studies.

ISSUES
None.

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PROJECT TITLE: Micromechanics of the Airway Smooth Muscle Cell

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT
The key end-effector of acute airway narrowing in asthma is contraction of the airway smooth muscle (ASM) cell. The key motor protein that drives ASM contraction is myosin. Both the myosin-based contractile apparatus and its cytoskeletal (CSK) scaffolding are dynamic structures that are in a continuous state of remodelling, but their dynamics are not well defined. This BRP is an interdisciplinary design-directed bioengineering project to fill that gap of knowledge. We propose to develop micromechanical technologies to measure cytoskeletal rheology, contractility, and remodelling. These technologies are based upon forced nano-scale motions of microbeads tightly bound to the cytoskeleton of the airway smooth muscle cell, spontaneous nano-scale motions of those same beads, and the relationship between them.

Taken together, these technologies comprise a suite of novel tools that is unequalled in its ability to characterize cytoskeletal mechanics at cellular and subcellular levels. From the point of view of clinical sciences, they have bearing upon the ASM cell and the role of that cell in bronchospasm, which is our stated goal. But these technologies will have bearing as well upon any integrative (patho) physiological process that has prominent mechanical components, including vasospasm, embryonic development, pattern formation, wound healing, crawling, metastasis, invasion, and mechanotransduction.

STATUS OF RESEARCH AND PARTNERSHIP
Our partnership has just gone through its competive renewal application and was succesful.

ISSUES
Bridging the life sciences and the physical sciences has made much headway in recent years. Our BRP is prospering, but within the broader institution there remains a cultural divide.

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PROJECT TITLE: Gene Therapy for Myocardial Stunning and Infarction

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT

    This purpose of this BRP was to apply an interdisciplinary bioengineering approach towards the study of a novel form of cardiac dysfunction identified during the course of a previous R01 project. These previous studies, performed in a murine model of reperfused myocardial infarction (MI), had confirmed that contractile dysfunction early after large myocardial infarction is not limited to necrotic tissue, but extends also to non-ischemic zones of the left ventricle (LV) remote from the ischemic region. We hypothesized that reactive oxygen species (ROS) and pro-inflammatory cytokines elaborated by leukocytes infiltrating the heart after reperfused MI might play a key role in the pathophysiology of this reversible form of LV dysfunction. We proposed that whole-animal experiments employing a complementary set of pharmacologic and genetic approaches would help to elucidate the role of inflammatory activation in remote zone LV dysfunction post-MI and to identify effective treatment strategies for preserving LV function after large MI. In preliminary studies, our partnership had already developed a mouse model of remote zone LV dysfunction and had validated it using cardiac magnetic resonance imaging (MRI). Using cardiac MRI in combination with molecular techniques, the functions of oxidative stress, TNF-a, NF-kB, and iNOS are now being evaluated using specific pharmacologic agents and genetically-manipulated mice. A multidisciplinary approach is employed that encompasses the fields of biomedical engineering, radiology, cardiovascular physiology, pharmacology, immunopathology, cell biology and molecular genetics. The specific aims are to:

1. Validate a novel cardiac MRI pulse sequence and use it to define the time course of remote zone LV dysfunction in mice. While our preliminary MRI studies showed that remote LV dysfunction resolves within 7 days after MI, we propose to apply a newly-developed CSPAMM-based DENSE pulse sequence to assess regional contractile function at even higher resolution.
2. Probe the pathophysiology of remote zone LV dysfunction post-MI using a pharmacologic approach. We hypothesize that pharmacologic agents capable of controlling oxidant stress, blocking TNF-a, inhibiting NF-kB and/or suppressing iNOS will preserve contractile function in remote, non-infarcted regions of the LV after large MI.
3. Probe the pathophysiology of remote zone LV dysfunction post-MI using genetic approaches. In preliminary studies, we have shown that contractile function in the remote LV is preserved in iNOS knock-out mice after large MI. Similarly, we hypothesize that remote LV function after MI will be preserved in TNF-a knock-outs, in mice with impaired NF-kB signaling, and in transgenic mice overexpressing SOD. Gene therapy with an Ad5 vector expressing SOD should yield similar results.

4. Determine the role of hematopoietic cells in remote zone LV dysfunction using bone marrow chimeras. We hypothesize that the beneficial effects of the genetic interventions investigated in Aim 3 may not depend entirely on hematopoietic cells, and propose a series of bone marrow transplantation experiments with iNOS knock-out mice to address this possibility.

STATUS OF RESEARCH AND PARTNERSHIP

The Partnership at UVA is successfully pursuing the Aims of the BRP. Technical information, methodological techniques, reagents and scientific insight are exchanged between the partners in an ongoing basis. The application of the CSPAMM-based DENSE pulse sequence in performing cardiac MRI in mice has been particularly rewarding. Dr. Epstein has successfully implemented this technique and has now extended it to characterize 3D myocardial mechanics in infarcted mouse hearts. The 3D characterization of myocardial mechanics using DENSE cardiac MRI in mice represents a significant advance, and we have recently published on this technique in the American Journal of Physiology (2005;288:H1491–H1497).

In the pursuit of Specific Aim 2, we recently tested the efficacy of a highly selective inhibitor of iNOS in the murine model of LV remodeling late after MI. The results of this pharmacologic study confirmed our previous findings in iNOS knockout mice showing that inhibition of iNOS dramatically reduces post-infarct LV remodeling. The results of this study were presented recently at the Annual Scientific Sessions of the Society of Cardiovascular Magnetic Resonance. With regards to Specific Aim 3, we have created chimeric mice by bone marrow transplantation (both iNOS knock-out mice reconstituted with wild-type bone marrow and wild-type mice with bone marrow from iNOS knock-out mice). Ongoing studies will determine whether it is the iNOS in bone marrow derived cells or tissue-resident cells that contributes to LV remodeling after myocardial infarction.

The ability to non-invasively assess contractile strain in the mouse heart over time after myocardial infarction has provided new insights into the pathophysiology of both remote zone LV dysfunction early after MI and LV remodeling late after MI. Thus tangible rewards have already resulted from this Partnership - in that scientific interactions have led to technical advances which, in turn, have yielded mechanistic insights into human cardiovascular disease.

ISSUES

In the Project Summary prepared for the Fourth BRP Grantee's Meeting, we identified an issue related to the aging 4.7T scanner upon which a great deal of the research in this BRP depends. We are happy to report that our proposal to the NCRR High-End Instrumentation Program for a new 9.4T MR imaging/spectroscopy system has now been funded. It is anticipated that this new MR scanner will support cutting-edge biomedical imaging research at UVA for many years to come.

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PROJECT TITLE: Development and Clinical Testing of CorAide RVAD/BVAD

PARTNERS' NAMES AND AFFILIATIONS:
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   Arrow International, Inc. (Reading, PA), Minnetronix, Inc. (St. Paul, MN)

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT
   The use of implantable left ventricular assist devices (LVADs) has been increasing to serve the growing population of patients with end-stage congestive heart failure. However, up to 40% of patients have significant right ventricular (RV) failure that limits the utility of implantable LVAD therapy. RV failure leads to two problems: decreased forward flow and high right heart pressures that result in passive congestion of the liver, kidneys, and abdominal organs. Both factors contribute to multiorgan failure, the leading cause of death after LVAD implant. Such patients commonly require prolonged inotropic support or support with a right ventricular assist device (RVAD). Clinically available RVADs are not intracorporeal devices and suffer from several limitations due to poor blood compatibility, high infection rates, poor long-term durability, need for anticoagulation, need for a hospital stay, high mortality, and a less than ideal quality of life. We have reported a poor prognosis in patients receiving LVAD support who also required external RVAD support or prolonged inotropic support. A safe, effective, intracorporeal RVAD could save the lives of many such patients with RV failure. We have developed the CorAide™ LVD-4000 Assist System, an implantable, third generation, centrifugal pump. A rotating assembly is fully suspended without mechanical contact or wear during operation. If the CorAide LVAD can be modified and used as an RVAD, the resulting biventricular assist device (BVAD) will be an ideal system for permanent support (destination therapy). The main objectives of this proposed program are to design, develop, and clinically evaluate an implantable RVAD that can be used as a component of an implantable BVAD for patients with severe biventricular failure. The specific aims are (1) Design and develop an implantable RVAD based on the CorAide LVAD, third generation centrifugal blood pump, (2) Design and develop an advanced fail-safe control algorithm capable of fixed speed or automatic mode that balances RVAD and LVAD performance, (3) Undertake in vivo characterization testing of the system both as an isolated RVAD and as a BVAD with the CorAide LVAD, (4) Undertake in vivo and in vitro reliability testing of the complete RVAD system, and (5) Obtain FDA approval for Investigational Device Exemption (IDE) and undertake clinical pilot studies using an institutionally approved program for patient selection and data collection.
   In this proposal, we will design and develop an RVAD in the first year, perform the characterization study in the second year, perform in vivo and in vitro reliability studies in the second and third years, and perform a clinical trail in the fourth and fifth years. The successful completion of this program will provide clinicians and patients with a safe and effective option for outpatient mechanical support that allows an excellent quality of life.

STATUS OF RESEARCH AND PARTNERSHIP

The initial phase of this program resulted in a prototype RVAD, named DexAide, a modified version of the CorAide LVAD. In vitro testing was performed in a stand-alone circuit as well as in a true RVAD mode to evaluate pump performance. Pump flow and power were measured under various afterload and pump speed conditions. The pump performance requirements of 2 to 6 L/min and a pressure rise of 20 to 60 mm Hg were successfully met with pump speeds between 1,800 and 3,200 rpm. The nominal design point of 4 L/min and 40 mm Hg pressure rise was achieved at $2,450 \pm 70$ rpm with a power consumption of 3.0 ± 0.2 watts. Normalized index of hemolysis was 0.030 mg/dl at 3,000 rpm (1.76 grams of hemoglobin/day) and 0.040 mg/dl at 2,200 rpm (2.28 grams of hemoglobin/day).

The development of a working prototype for the stand alone RVAD Portable Electronics Module (RPEM) and RVAD System Interface (RSI) were also the focus of the first stage of the BVAD BRP program plan. The CCF Fixed Flow control algorithm was developed to match the RVAD pump flow to a clinician programmed target flow. The algorithm includes a method to detect ventricular unloading and prevent ventricular suction based on pump flow response to fixed speed increments.

Four acute in vivo tests confirmed acceptable pump performance in variety of conditions, such as low circulating volume, high contractility, high pulmonary artery pressure, vasodilation, and low contractility. While planned short-term (2 weeks) in vivo tests (4 calves) were complicated by partial occlusion of the calf inflow cannula, reasonable biocompatibility of the pump without the use of heparin was demonstrated. The Fixed Flow control algorithm worked well as designed. The calf inflow cannula is being redesigned, and chronic in vivo tests and evaluation of the external electronics are underway.

The strong partnership developed during a separate program continues to provide fruitful results. The extensive use of meetings, teleconferences, and e-mail enhance communication between the partners.

ISSUES

Partial obstruction of the calf inflow cannula has been a problem and necessitates further design refinement. Otherwise, no major issues have been encountered, and we are pleased with the progress made in the initial year of this BRP.

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PROJECT TITLE: Molecular Analysis of Visual Processing

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GRANTING NIH INSTITUTE/CENTER: National Eye Institute

ABSTRACT
We propose to develop a set of molecular tools to link gene expression to, and to study the role of specific neural circuits in, visual perception and behavior. These tools will be adapted for work in non-human primates, which have distinct advantages in our knowledge of the functional anatomy of neural circuits, the functional architecture of cortex, the ability to study complex behaviors and to combine physiological and behavioral studies in awake, behaving animals, and because of their close relationship with humans. The components of the project include developing gene microarrays based on an expression library derived from the monkey cerebral cortex, high throughput techniques for studying patterns of gene expression involved in specific behaviors, anatomical studies of the patterning of gene expression relative to cortical functional architecture and cell type, developing viral vectors for delivering genes to neurons, reversible inactivation of specific cell classes using molecular tools, and a cortical plasticity model for monitoring changes in gene expression and altering function by changing levels of gene expression via viral transfection. Once developed, these techniques will make possible a top-down understanding of the link between patterns of gene expression and behavior. They will also make it possible to alter gene expression in higher animals for the study of neural mechanisms of behavior.

STATUS OF RESEARCH AND PARTNERSHIP
Monitoring and manipulating gene function in monkey visual cortex. We refined the printing of the monkey visual cortex gene expression array, and are now using it to follow changes in gene expression following retinal lesions. A major advance in this effort was to join laser capture microscopy (LCM) with the microarray work, to address the problem of masking changes in gene expression because of the diversity of cell types in the brain. A second area that we have developed in the last year was the application of RNA interference (RNAi) to manipulating gene function in monkey visual cortex.

Genetic methods for inactivating visual pathways. This entails using the drosophila allatostatin receptor (AlstR) system for reversible neuronal inactivation in vivo. We used adeno-associated virus serotype 2 (AAV-2) carrying the AlstR gene to infect LGN neurons in monkeys and in ferrets. These experiments showed that neurons expressing the receptor could be quickly and reversibly inactivated following administration of allatostatin (AL) close to the LGN. We extended the system to other brain regions, particularly the cerebral cortex. Both of these goals were facilitated by the development of pseudotyped AAV vectors.

Viral vectors for gene delivery. In addition to testing multiple pseudotypes of AAV and also VSVg pseudotyped lentivirus for their ability to infect and drive transgene expression in the primate LGN, cortex, and amygdale, we have had success in using another approach for cell type specific targeting, using a lentivirus pseudotyped with the envelope protein for rabies virus (rather then the typical VSV pseudotype). We found that this virus can infect neurons selectively through their axon terminals, allowing specific cell types to be targeted based on their unique extrinsic projections. We have also used herpes simplex virus (HSV) amplicon vectors, which, like AAV and lentivirus, are replication incompetent and can give stable long term gene expression without toxicitiy, but which have a much

larger capacity allowing promoter sequences up to at least 120kB, permitting the use of larger promoter sequences than possible with AAV or lentivirus.

ISSUES

The outstanding issues are essentially the challenges represented by the project itself, but ones for which progress is continually being made: developing the most effective means of detecting differential gene expression in a complex tissue with a wide diversity of cell types, manipulating gene expression to alter the course of normal brain processes, developing viral vectors and promoters that provide cell type specific gene expression, and delivering drugs to neurons expressing receptors delivered by the viral vectors.

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PROJECT TITLE: Microchip Devices To Assay Quantal Exocytosis

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GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke

ABSTRACT
Peptide hormones and neurotransmitter are stored in membrane-bound vesicles within endocrine cells and neurons. Upon stimulation, a rise in intracellular Ca2+ concentration triggers the fusion of vesicles with the plasma membrane and release of hormone or neurotransmitter to the outside of the cell in a process called exocytosis. Since fusion of each vesicle discharges a discrete packet of signaling molecules, exocytosis is inherently a quantal process. The objective of this BRP grant is to develop microdevices for high-throughput electrochemical measurement of quantal exocytosis from neurons and neuroendocrine cells. Our goal is to develop sophisticated devices that can assay quantal exocytosis from thousands of cells in a day and thereby greatly accelerate the pace of basic neuroscience research. In addition, this technology will enable high throughput discovery of drugs such as L-DOPA (used to treat Parkinson's disease) that affect quantal exocytosis and screening for toxins such as tetanus that inhibit neurotransmitter release. Our Specific Aims are to 1) Target individual cells to electrochemical microelectrodes on microfabricated devices, 2) Develop approaches to stimulate exocytosis from cells on microdevices, 3) Integrate carbon-based electrochemical electrode materials into microdevices to increase sensitivity and performance, and 4) Develop electronic instrumentation to allow simultaneous recording of many channels of electrochemical or electrophysiological data.

STATUS OF RESEARCH AND PARTNERSHIP
Our first year research activities are summarized below.

1. We are testing a soft lithography approach to fabricate microfluidic channels to transport cells to specific docking sites on microchips. The polymer polydimethylsiloxane (PDMS) is molded over patterned photoresist and then sealed to a glass substrate. Multiple layers of photoresist were patterned on the "master" to mold PDMS microfluidic channels of different heights. Thicker lines produce microfluidic channels deep enough for cells to pass whereas the thin layer produces orthogonal channels that allow solution to flow yet are too shallow to allow cells to pass. After molding of PDMS on the master and sealing to a glass substrate, the device is complete. Cells were injected into the inlet reservoir connected to one of the deeper channels with a syringe pump. The only path for fluid movement is through the shallow channels to the deep "exit" channel that is connected to a waste reservoir. Using this approach, we have successfully trapped cells at the inlet to the shallow vertical channels. We are working on extending the design using microfluidic valves so that cells can be flushed from trapping sites to enable multiple rounds of experiments on the chip.

Once the cell trapping approach is developed to a satisfactory level we will begin the process of integrating the electrochemical electrodes into the docking sites.

2. We are using an optical approach to measure the rate of solution exchange around a cell passing through a fluid junction in a PDMS microfluidic device in anticipation of using this approach to rapidly stimulate exocytosis from cells. First, we labeled the surface membrane of cells using the fluorescent styryl dyes FM1-43 and FM2-10. These dyes fluoresce when inserted into membranes, but are essentially non-fluorescent in free solution. Labeled cells flow through a microfluidic channel where they encounter a junction with a channel containing dye-free solution. As each cell passes through the fluidic junction, a rapid drop in the concentration of dye surrounding the cell occurs and the fluorescence of the cell decays as the dye departitions out of the surface membrane. We found that the decay in FM2-10 fluorescence occurs with a nearly exponential time course with a time constant of less than 50 ms, which serves as an upper bound for the time it takes to exchange the solution surrounding the cell.

3. We are using two approaches to fabricate carbon-based electrodes: 1) self-assembly of carbon-based nanoparticles (boron-doped nanocrystalline diamond, carbon nanotubes and graphite nanoparticles) in a silica matrix, and 2) deposition of nitrogen-doped DLC (diamond-like carbon) films by PECVD or sputtering to get conductive carbon-based films.

In a different approach we are testing Indium Tin Oxide (In_2O_3/SnO_2, ITO) as a potential electrode material for measuring quantal exocytosis because it exhibits excellent optical transparency, high electrical conductivity, a wide electrochemical working window, and ITO films on glass substrates are readily available. A transparent electrode is desirable because it allows one to easily visualize cells as they are tested and allows concurrent optical measurements and manipulations, e.g., fluorescent Ca^{2+} measurements, visualization of expression of Green Fluorescent Protein in cells and photorelease of caged Ca^{2+}. We have successfully tested ITO electrodes with both simple analytes and with actual cells.

4. We designed and assembled 2 types of amplifier modules using off-the-shelf components that will enable simple and economical implementation of dozens of amplifiers in parallel. The first design is a 3-electrode amplifier suitable for passing large currents such as when performing basic characterization of new electrochemical electrode materials. The second design resembles that of a simple patch-clamp amplifier in that it has only 2 electrodes, a feedback resistor, and a high-performance op-amp with low input bias current. This latter design is appropriate for recordings from microelectrodes. We have fabricated a prototype and are currently testing the performance of this device.

The other approach we are pursuing is developing CMOS technology to enable hundreds or thousands of amplifiers to be placed on a single chip. We fabricated a test chip in a submicron CMOS technology through the Metal-Oxide Semiconductor Implementation Service (MOSIS), containing individual devices and amplifiers to characterize the properties of our "shared amplifier" circuit experimentally. We also designed a 5x5 array of 9umx9um electrodes on the test chip. The top metal layer in the CMOS chip is aluminum. To detect quantal exocytotic events, we will use platinum electrodes. We plan to deposit platinum on the aluminum electrodes at the Cornell Nanofabrication facility (CNF). We will then conduct experiments with cells on top of the electrodes.

ISSUES
We have no significant issues to report.

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PROJECT TITLE: MEMS Sensors for Arrhythmia Detection and Intervention

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT

Despite decades of intensive investigation, sudden death secondary to ventricular fibrillation (VF) remains a leading cause of mortality in the US and other developed countries. Recently, several promising hypotheses regarding the mechanism for VF have been introduced. However, it has not been possible using currently available experimental techniques to determine which theory (or theories) is most applicable to VF. To address this issue, we propose to: 1) construct a cardiac mapping system from nanofabricated components that is capable of assessing cardiac activation and repolarization with high spatial and temporal resolution and with minimal tissue damage; 2) use a novel phase mapping technique to analyze the mapping data, with the objective of identifying the location and number of phase singularities during sinus rhythm, ventricular tachycardia and VF; 3) use the phase singularity data to distinguish between three putative mechanisms for VF – an anchored rotor with fibrillatory conduction, a meandering rotor or multiple rotors. MEMS technology will be used to construct microscale mechanical needle-like structures with integrated electrodes that are ultrasonically activated, to minimize tissue damage during insertion. The electrode arrays will be used to map activation and repolarization in canine ventricular myocardium in vitro and in normal and acutely ischemic pig hearts in situ during fixed pacing and during VF. The mapping data will be analyzed using a fast Fourier-demodulation technique to identify singularities and wave vectors during VF. Computer models of 2- and 3-D myocardium also will be used to generate surrogate data sets for testing the analysis algorithms. The results of this study will lead to significant advances in three key areas: development of devices to map cardiac electrical activity with unprecedented spatial resolution; application of newer and more sophisticated techniques to analyze large mapping data sets; interpretation of high resolution mapping data within the context of novel hypotheses regarding the genesis of ventricular tachycardia and fibrillation.

STATUS OF RESEARCH AND PARTNERSHIP

We have developed ultrasonically actuated silicon thin microprobes that successfully penetrate canine cardiac tissue in vitro. Both the design and the fabrication of the probes are similar to ultrasonic surgical tools developed previously by the sonicMEMS group at Cornell University. The process flow is as follows: a 6000 °A LPCVD silicon nitride is deposited on 4-inch <100> silicon wafers as electrical insulation and wet-etch mask layer, after which a 3000 °A Pt/300 °A Cr layer is evaporated and patterned to form the electrode arrays, interconnects, and bonding pads. A 1 μm PECVD silicon nitride insulation layer is deposited on top of the metal layer and a second layer of Pt/Cr is evaporated and patterned to form a ground layer to reduce cross-talk. A 1 μm PECVD silicon nitride passivation layer is deposited and openings are etched for the electrode recording sites and bonding pads. A DRIE etch on the front side of the wafer defines the shape and depth of the two microprobes at the tip of the ultrasonic horn. Finally, a backside-only KOH etching produces the silicon ultrasonic horn with the microprobes at the horn tip.

The catenary shaped silicon ultrasonic horns are 6 cm long, 1 mm thick, with end-to-tip area ratio of 15 mm2 to 1 mm2. Two thin-beam silicon microprobes are patterned at the tip of the horn, with a 300 μm space in between. Microprobes with 5 mm length, 100 μm to 200 μm width and 60 μm to 140 μm thickness have been fabricated and tested. There are five 40 μm x 40 μm Pt/Cr electrodes 1 mm apart on each of the two microprobes, forming a 2 by 5 electrode array. The microprobes are excited at their base by the ultrasonic horn, with longitudinal displacement amplitude of 0.37 μm per Vpp driving voltage. To reduce the excitation force in transverse directions, another silicon horn with same shape and dimension, but without the two microprobes is bonded to the horn with microprobes to achieve symmetry in thickness direction. Two piezoelectric PZT-4 plates with matching resonance with the silicon ultrasonic horn are bonded to both sides of the horn at the half-wavelength displacement node. The horn is then clamped to a custom made PC board, also at the location of the displacement node. The Pt/Cr pads on the probe are wire-bonded to the PC board and then connected to an external circuit through a ribbon cable.

Ultrasonic actuation of the microprobes reduced penetration force by 4% per Vpp driving voltage from 2 Vpp to 13 Vpp, and allowed the probes to be inserted into cardiac muscle without breakage or significant buckling. The probes have been used to record electrophysiological signals from multiple sites simultaneously within the heart wall. Cardiac signals have been recorded from isolated perfused canine ventricles during pacing, following the induction of ventricular tachycardia and during the transition from ventricular tachycardia to ventricular fibrillation. These recordings can provide information for reconstruction of the electrical wave propagation within the heart that is crucial to understanding the mechanisms for lethal cardiac arrhythmias.

ISSUES

Several obstacles remain to be overcome before the probes can be used to obtain high resolution 3-D maps of cardiac excitation, including: 1) validation of the stability of the electrical recordings obtained by the probes; 2) assessment of the damage caused by probe insertion; 3) a scaling up of the probe fabrication and characterization processes so that a large number of probes are available. Once the mapping data are available, they will be analyzed and compared with the results of on-going computer simulations using anatomically realistic ionic models of the canine ventricle.

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PROJECT TITLE: Role of Biopolymers and Lipids in Kidney Stone Formation

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GRANTING NIH INSTITUTE/CENTER: National Institute of Diabetes and Digestive and Kidney
Diseases

ABSTRACT
 The objective of the proposed bioengineering research partnership (BRP), located at the University of
Florida, is to examine two key issues relevant to urolithiasis; 1) the effects of acidic biopolymers and lipid
membranes on nucleation, growth and aggregation of calcium oxalate (CaOx) and phosphate (CaP)
crystals in an artificial urinary environment; and 2) the injurious effects of a liquid-phase mineral
precursor on tubular epithelial cells grown in culture. With regard to 1), many investigators have
examined the promotory and inhibitory effects of acidic proteins on crystal growth and aggregation. More
recently, the biomineralization community has turned their attention to the occurrence of amorphous
mineral precursors. Our work here is focused on the relevance of polymer-induced liquid-precursor
(PILP) phases to pathological biomineralization, because the PILP process generates non-equilibrium
crystal morphologies which exhibit features similar to crystals found in kidney stones, such as for
example, stratified spherulites. In addition, the interfacial aspects of this liquid-liquid phase separation
process lead to a pronounced aggregation tendency of crystals. Lastly, we hypothesize that the presence
of this cementatious mineral precursor in the urinary tract could influence the attachment and retention of
crystals to renal epithelial cells; or the highly ionic precursor phase could cause cell injury or death,
leading to the release of modulatory factors or membrane fragments, which could promote heterogeneous
nucleation and/or aggregation of crystals. The proposed work consists of 10 Specific Aims which fall
under four topical areas describe in the section below. The bioengineering techniques include
measurement of interparticle forces by AFM, measurement of long-range interactions between submicron
CaOx particles and mimetic lipid membranes with an optical trap force transducer, and nucleation of
crystals and PILP phase on mimetic lipid membranes using Langmuir monolayers. This 4-year project
will enable us to assess the role of lipids and acidic biopolymers in stone formation, and will contribute to
the development of bioengineering techniques that are new to the field of stone research.

STATUS OF RESEARCH AND PARTNERSHIP
1. *Crystal-Macromolecule Interactions:* The effects of mimetic proteins on crystal nucleation and
   growth, with emphasis on the ability to generate a PILP phase in either the CaOx or CaP systems, is
   being examined. In last year's report, Gower's group demonstrated that a polymer-induced liquid-
   precursor (PILP) phase can be generated for the CaP system. This year, we have been optimizing this

system using the Constant Composition apparatus purchased with this grant, to produce spherical amorphous particles of CaP which are being used in the other studies described below.

El-Shall's group has been using statistically designed experiments to examine the effect of Supersaturation, Hyperoxaluria, Hypercalciuria, and Citrate on Nucleation & Crystal Growth of Calcium Oxalate Monohydrate. For a better understanding of the primary nucleation and kinetics of growth of COM crystals, the interfacial free energy was determined using induction time measurements. The experimental design results correlate with theoretically anticipated trends.

2. *Crystal-Crystal Interactions:* Kidney stones frequently contain a calcium phosphate spherulitic core, surrounded by aggregates of calcium oxalate crystals (along with organic matrix). Although spherulites are considered aggregates of crystals, their formation mechanism is entirely different from the aggregation of preformed crystals. Both types of aggregates are seen in stones, but the mechanism of spherulite formation (which serves as the nidus of many stones) has largely been ignored in the literature. Gower's lab has been examining the formation of spherulites which frequently occur in the PILP process, as well as deposition of CaOx crystals onto amorphous calcium phosphate (ACP) particles formed via the PILP process. The stages of formation of these CaP-CaOx composite aggregates, which mimic the microstructure of mixed composition stones, are being examined.

4. *Crystal-Cell Interactions:* Rabinovich's lab has focused on AFM measurements of the interaction forces between Calcium Oxalate Monohydrate (COM) crystals and a monolayer of renal epithelial cells. The effect of prior treatment of cells by oxalate solutions was investigated. Adhesion forces have been measured for COM/MDCK interaction, while no adhesion was observed for COM/ LLCPK1-cells. This difference correlates with the in vivo situation that kidney stones form primarily on MDCK-cells and not on LLCPK1-cells. On the other hand, further studies are needed to determine if the adhesion force between COM and MDCK could be related to the specific interaction of oppositely charged COM crystals and phospholipid/ phosphate complexes in the cell.

Khan's group has been examining the involvement of reactive oxygen species in calcium phosphate induced renal epithelial cell injury: Calcium phosphate (CaP) is most likely to form in the early segments of the nephron and can nucleate CaOx in a metastable solution. To test the hypothesis that CaP can injure the renal epithelial cells, we exposed proximal tubular origin LLC-PK1 and collecting duct origin MDCK cell lines to various concentrations of Brushite (Br) crystals and examined various biochemical indicators of cell injury. It was found that Br crystals are injurious to cells of both the proximal tubules as well as collecting ducts, and injury is a mediated by reactive oxygen species. We propose that CaP crystals can independently interact with renal epithelium, promote sites for crystal attachment, and then either grow into a CaP stone or create sites for CaOx crystal nucleation, retention and stone development.

3. *Crystal-Lipid Interactions:* Talham's lab uses Brewster Angle Microscopy (BAM) to monitor the COM precipitation at phospholipid monolayers that are models of cell membranes. Research efforts have been focused on the role of phase boundaries on heterogeneous COM precipitation. Liquid expanded (LE) and liquid condensed (LC) biphasic monolayers can be formed from either a single lipid or a mixture. Control of the phase behavior can be afforded by changing the monolayer composition and degree of compression. The data thus far suggests that COM precipitation can only occur at a phase boundary if there is a dynamic exchange between the molecules in the LC and LE phases.

Dickinson's lab has applied their optical trap force measurement technique to determine equilibrium forces between amorphous micron-sized calcium phosphate PILP particles and a phospholipid (DPPC) bilayer. Force measurement results obtained at low electrolyte concentration suggest that the difference in the electrostatic potential profile between a bare mica surface and a DPPC bilayer coated surface is small and that the long-range double layer forces prevent contact between the particle and bilayer. At high electrolyte, force measurements of a PILP particle interacting with a bare mica surface in 10 mM NaCl have shown no attachment, while surfaces coated with a DPPC bilayer enhance the attraction of a calcium phosphate PILP particle and the likelihood for attachment to occur.

ISSUES

No problems - the interdisciplinary team provides for very stimulating discussions during our biweekly meetings and has allowed for new research areas to be explored which we could not have done individually.

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PROJECT TITLE: Magnetic Resonance Guided Electrophysiology Intervention

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT

Ventricular tachyarrhythmias and atrial fibrillation occurring in patients with structurally abnormal hearts are the most important arrhythmias in contemporary cardiology, and despite much progress, remain therapeutic challenges. Invasive electrical studies of the heart (electrophysiologic studies) are often used in the diagnosis and therapy of arrhythmias, and many arrhythmias can be cured by selective destruction of critical electrical pathways with radiofrequency (RF) catheter ablation. A major limitation in studying arrhythmias in patients, however, is the lack of ability to accurately correlate anatomical and electrical information. Another major limitation is the lack of ability to visualize ablated areas of myocardium during catheter ablation procedures, making it difficult to confirm the presence of ablated lesions in the desired locations. We are developing ways of combining the anatomic information from magnetic resonance imaging (MRI), with electrophysiologic testing and ablation.

We hypothesize that MRI, with non-magnetic electrode catheters, tip-location sensors, intracardiac receivers, real-time MRI scanner control, remote-control catheter manipulators, and 3-dimensional imaging software can (1) provide the ability to accurately visualize cardiac anatomy, (2) provide accurate navigation of catheters without radiation, (3) provide the ability to visualize ablated lesions, and (4) aid in producing more accurate electrical maps. Our initial 5-year project dealt with (1) technology development, (2) demonstration of the feasibility of MRI guidance of catheters in animals, and (3) lesion visualization in animals, and in patients with atrial arrhythmias. Our 5-year continuation project will deal with (1) additional technology development, (2) improved integration of the different subsystems, (3) study of the determinants of successful ablation in patients undergoing standard ablations, and (4) broadening of the applications to real-time MRI guided therapy in patients with atrial and ventricular arrhythmias. The technologies developed in this project, should, in addition, be applicable to using MRI to guide interventional procedures in general.

STATUS OF RESEARCH AND PARTNERSHIP

The major accomplishments of the project include: (1) Demonstration of the feasibility of using MRI to guide interventional procedures in the heart, (2) Development of a clinical-grade catheter system for performing electrophysiologic procedures in patients, (3) Approval of an Investigational Device Exemption by the FDA (IDE #G010093) for testing the clinical grade system in patients, (4) Installation of new generation interventional MRI scanners with substantial real-time capabilities at our institution, (5) Substantial improvement in real-time image processing capabilities, and (5) Approval of the competing renewal of the project for another 5 years.

We have added an additional partner, the Johns Hopkins Imaging Institute, to enhance our real-time imaging software. The institute has expertise in real-time imaging, and has undertaken the task of

processing images in real-time to isolate regions on interest to remove overlaying structures that obscure visualization (segmentation), as well as registering multiple images upon each other. Algorithms are being developed for superimposing lesion images, acquired with acquisition times of a few seconds (not suitable for real-time guidance), on the real time images acquired with acquisition times of less than 0.1 second (suitable for real-time guidance). This superposition allows for the presence of real time targets, and is especially useful in filling in gaps in lines of lesions.

Our Investigational Device Exemption covers catheters to be used with low-power MR scans. Safety data shows that standard low power MRI pulse sequences do not cause unacceptable heating of those catheters. To optimize imaging, we are also developing clinical grade catheters that can be used with the highest power MRI pulse sequences. Substantial progress has been made on these improved catheters. A new filtering topology has been developed that allows miniaturized filters to be incorporated into the electrodes to optimize filtering and allow very high-energy MRI sequences to be used.

Other technological developments are continuing. We are developing a new technology for the tip location system, since testing has shown that the current technology, based on reading the gradient fields of the scanner, are limited in noise rejection. It has proved very difficult to have sensors manufactured that have adequate signal-to-noise ratio for consistently locating the tip within the specified 1 mm. The new technology is based on having a series of external, decoupled transmitter coils, which emit a low frequency, low intensity magnetic field. The magnetic field frequencies are detected by the catheter tip sensors, and position is determined in an analogous way as with global positioning systems. A theoretical analysis has already been performed, validating the concept. Sensors using this new technology can be made smaller and require less precision in construction, than sensors built for the gradient-field-based technologies.

Several new MRI scanners are being installed at Johns Hopkins University/ Hospital. The University/Hospital has decided to switch from scanners supplied by GE Medical to scanners supplied by Siemens. We have already started implementing routines for transferring image data from the Siemens scanners to our imaging console. The imaging console performs 3-dimensional reconstructions of the 2- dimensional imaging data in real time, and allows for rotation and opening of the image in arbitrary planes and angles in real time. This image manipulation capability allows for optimal viewing of the cardiac structures for guidance of interventional procedures.

We have also made progress on an MRI compatible remote catheter manipulation system. This system will allow any MRI scanner to be used for guiding catheter-based procedures. A working system has been produced, consisting of wheels and gears that can advance, withdraw, and rotate a catheter. The mechanism is being changed to plastic to make it MRI compatible. MRI compatible actuators, based on piezoelectric materials, have been identified, which will produce the forces for moving the catheters.

We have imaged 28 patients with non-ischemic cardiomyopathy, prior to defibrillator implants, to assess the degree of scaring to study the substrate for ventricular tachycardia. We are shifting emphasis of our imaging studies to patients with ventricular tachycardia to include that very important disease entity. We have noted that some patients with non-ischemic cardiomyopathy have intramyocardial scar, and sought to determine if scar was the substrate needed for ventricular tachycardia, as evidenced by inducibility at electrophysiology study (EPS). Scar morphology was detected by contrast enhanced MRI, and inducibility was determined by EPS. We noted that scar was often non-transmural, and divided each MRI image slice into 12 segments and noted the average scar transmurality in each sector. By the Wilcoxon rank-sum, the only MRI predictor of inducibility was the presence of average scar transmurality in the 26-75 percentiles (p=0.005). Non-transmural scar, thus appears to be the substrate for ventricular tachycardia in non-ischemic cardiomyopathy. This type of analysis may lead to identification of patients who are candidates for intervention, including MRI guided intervention.

ISSUES

We have added and deleted partners, and are pleased with the flexibility of the BRP, as these changes have enhanced the overall program. We feel that the ability of BRPs to develop new technologies as a primary goal has accelerated substantially the development of these MR-guided interventions.

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PROJECT TITLE: Imaging Structure and Function in Small Animals

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT

This bioengineering research partnership will develop a dual-modality CT/SPECT system for high-resolution imaging of radionuclides in transgenic and knockout mice that now are in widespread use to model the mechanism, diagnosis, and treatment of human diseases. This research will be focused on the development of techniques that correlate structure and function, and that can perform noninvasive and quantitatively accurate measurement of tissue metabolism and organ physiology in small animals using radiolabeled tracers. Within this context, the re-search program includes 5 specific aims. (1) A pinhole SPECT system will be developed using a pixellated solid state detector (cadmium zinc telluride) for radionuclide imaging of small animals using 125I (27.5 keV), 99mTc (140 keV), and other radionuclides. (2) The pinhole SPECT system from Specific Aim 1 will be integrated with a cone-beam computed tomography system volume to allow sequential acquisitions of CT and SPECT images without moving the animal. (3) Cone-beam tomographic algorithms will be implemented for reconstruction of the radionuclide and x-ray tomographic data from the small animal imager. Techniques will be developed that use the re-constructed CT and SPECT data to quantify regional distribution of radionuclide concentration at spatial resolutions suitable for mice. (4) The dual-modality imaging system will be used for in vivo measurement of cardiovascular physiology in transgenic mice to investigate the role of the sym-pathetic innervation in heart disease. These measurements will test the hypothesis that increased heterogeneity of sympathetic innervation is related to the development of congestive heart failure. (5) The dual-modality imaging system will be used to measure the tumor and organ distribution of humanized anti-HER2 monoclonal antibody in a transgenic mouse model of metastatic breast cancer. The overall goal of this project will develop a high-resolution imaging system that com-bines CT and SPECT to correlating structure and function. The system also will be designed to perform noninvasive serial studies in mice, and to replace invasive direct tissue sampling and autoradiography for biodistribution studies and functional assessments using radiolabeled tracers in transgenic mice.

STATUS OF RESEARCH AND PARTNERSHIP

We had completed the hardware and instrumentation development phase of the project (Specific Aims 1-3), and we are moving to the application phase of the project (Specific Aims 4 and 5).

A major effort in collaboration with our partner Jamco Engineering has been devoted to the design and production of a gantry for the imaging system. The gantry includes the detector enclosures, a slip ring, animal bed and its linear motion mechanism, and the supporting mechanical parts for the readout electronic components. All of the hardware and instrumentation components have been completed, and

we now are performing the final integration of the system. We expect the mechanical accuracy of the system to be maintained within 10 microns with an angular positioning accuracy of approximately 6 arcsec.

The development of microSPECT subsystem using cadmium zinc telluride (CZT) detectors, in collaboration with our partner Photon Imaging, Inc., has been finalized. Two completed CZT detectors were tested at UCSF using several radioactive sources (99mTc, 111In, 57Co, 109Cd, 125I, and 241Am). These CZT detectors have demonstrated excellent energy resolution (e.g., 6.5% at 140 keV) and detector quality with less than 1% bad pixels. The gamma cameras have been tested, showing a sensitivity of 5×10^{-4} at a rotation radius of 25 mm, and a spatial resolution of 700 µm. The microCT subsystem (a low-power microfocus x-ray tube and a high-resolution CCD camera), also in collaboration with Jamco Engineering, has been integrated at UCSF, and demonstrated an imaging capability with up to 50 µm spatial resolution using a benchtop test setup.

At UCSF, we have developed both analytic (Feldkamp) and iterative (maximum-likelihood expectation-maximization) software for reconstructing tomograms from the microCT and microSPECT data. We also developed methods to correct the radionuclide data for photon attenuation using an attenuation map derived from the correlated microCT data, and for the geometrical response of the radionuclide collimator. For the microCT, we have developed software to correct image distortions that are introduced by the optical taper of the CCD x-ray detector. As a result, with the use of correction techniques, our microCT system demonstrated a volumetric resolution of 70^3 µm in the entire field of view.

We currently are developing methods of helical scanning and cardiac gating for the microSPECT subsystem. The required software for reconstructing tomograms from helical scanning has been developed, and currently under evaluation. The hardware component and software for the cardiac gating are being developed. Our preliminary studies using phantoms show that our microSPECT subsystem can perform to provide sufficient image quality for in vivo cardiac-gated myocardial perfusion studies using mouse models.

ISSUES

The complete system is being finalized as of July 2005. A final system evaluation will be performed to have a complete characterization of the instruments.

Our original BRP proposal called for two specific studies involving small animals that we intend to pursue after we complete the development of the imaging system. In the first, we will perform dual-isotope studies to correlate adrenergic innervation (with 125I-MIBG) and myocardial perfusion (with 99mTc-MIBI) with transgenic mice that express congestive heart failure: In the second, we will measure tumor and organ distribution of HER2/neu antibody radiolabeled with 125I, in a mouse model that overexpresses HER2/neu. We expect to perform these studies during the final year of the grant, with the goal of evaluating the SPECT/CT system for murine models of cardio-vascular disease, cancer, and other diseases. These animal models are still available in addition to several other models from investigators with whom we are in the early phases of establishing collaborations. These include use of small animal SPECT/CT to assess the distribution of 111In-liposome-based drug delivery and for which we have pilot data showing a spatial resolution of 750 µm in imaging tumor uptake of the radiolabeled liposome. An in vivo imaging study to evaluate therapeutic response of metaiodobenzylguanidine (MIBG), an adrenergic analog, labeled with 131I in a murine model of neuroblastoma is also in its preliminary phase. Other studies include in vivo functional assessments in response to stem cell therapy in a mouse model of myocardial ischemia and infarction, and following the progression of metastasis in a mouse model of lung cancer. In the coming year, we plan to develop the capabilities of SPECT/CT for these and other in vivo studies involving small animals using the system developed within this BRP.

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PROJECT TITLE: Quantitation of Cellular Protein Production in Real Time

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT
This partnership focuses on development of automated technologies for gathering and interpreting information about marker and secreted protein expression at the individual cell level. Our biomedical research partnership combines the technological expertise of Automated Cell, Inc. (ACI) with the biomedical expertise of researchers at University of Pittsburgh Cancer Institute (UPCI) and clinical associations at Harvard Medical School (HMS) to focus on the development of tools for real time study of individual cells and mixed populations of cells in an automated combinatorial cell culture system.

Goals include: 1) Development of software for real time analysis and dynamic manipulation of osteoblast progenitor/precursor cells; 2) Development of devices and procedures for coherent real time detection of phenotypic changes in rare human cells and the progeny of such cells deposited, tracked, imaged and analyzed individually through time; 3) Innovation of technologies for real time quantitation of cellular protein production through miniaturized assay methods and off-line analytical systems; and 4) Application of these technologies to determine effects of aging on osteoblast differentiation potential of human bone marrow stromal cells, utilizing the STRO-1+ subset, in an in vitro model system.

STATUS OF RESEARCH AND PARTNERSHIP
In the third year of this partnership we have continued to develop technology for automated live cell analysis and have begun to expand our investigation of osteoblast cellular biology to include the role of the osteoblast in the hematopoietic stem cell niche (Bahnson et al 2005a). Progress has continued in the application of fluidics, improvements to the functionality of the microscope and associated cell culture environment, and software innovation including implementation of a workflow program meeting our goal for real time analysis. These efforts are continuing in coordination with those at UPCI, where induction of differentiation in osteoblast-type cells and primary bone marrow STRO-1+ cells from hip replacement patients at HMS are being analyzed. Additional manual and statistical validation was required for completion and acceptance of our study of osteoblast cell response to extracellular matrix components, and this paper now represents a comprehensive description of the automated methodology that we are bringing to bear in this project (Bahnson et al 2005b).

A milestone was recently reached in software integration such that microscope images are automatically and immediately handled by the image processing software, incorporating automated exportation of the numerical data into the database. Since results in the database can be monitored and updated in real time, the final link to attaining the goal of dynamic manipulation of experiments now requires development of the feedback component that enables the user to set criteria within database queries that access real-time results. When desired criteria are met, this component will then initiate procedures at the microscope/fluidics workstation to stain specific wells, exchange medium, add components, and/or modify image acquisition settings as appropriate, for example, to determine the

fluorescence marker intensity after staining for assessment of cellular differentiation or for any number of other possible interventions. A challenge of our technology lies in the wide array of analytical options that are possible.

For fluorescence auto-focusing, we have more carefully defined requirements and specified user options that provide desired features for this project and we are currently integrating these into the system operating program. The image features for analytical fluorescence quantitation are also being incorporated into the data processing software to compensate, for example, for background fluctuations with lamp variability and variability in the interference from medium and residual dye components after staining, and for correlating fluorescence intensity of cell surface markers with cell position across the view-field. We did not fully anticipate the requirements for accurate fluorescence focusing and other details related to fluorescence quantitation at the individual cell level that are crucial for accuracy. To further streamline our approach to optimal focus, we are using 2um fluorescent beads and have characterized limits to plate surface variability. Since we are seeking to apply fluorescence to live cell imaging over long time periods, our goal during auto-focus is to minimize the fluorescence exposure of cells that is associated with photo-bleaching and photo-toxicity.

We have continued to incorporate design features that make the new system compatible with robotic removal and replacement of plates for higher throughput and for coordination of plates from simultaneous long-term and short-term experiments. This design also provides the option for using fluidics in a remote location, in which case the fluorescence auto-focus features discussed above will become even more crucial.

ISSUES

Primary bone marrow cells from patients at HMS continue to present challenges related to extreme low contrast and inter-patient variability in seeding efficiency and viability. We are pursuing a variety of approaches for extracting useful data, including manual methods when necessary for measuring motility and growth characteristics. In the meantime, and in light of recent indications that osteoblasts are crucial players in hematopoietic stem cell (HSC) niches (Moore 2004), we have begun to examine cell-cell interactions between HSC and osteoblast/stromal cells as a part of this project. We feel that the unique capabilities of our ultimately integrated system will be particularly applicable to temporal resolution of differentiation among mixed cell types in so called "cellular niches."

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PROJECT TITLE: Histo-Mechanics and Biology of Remodeling in Hypertension

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ABSTRACT
Hypertension remains a major risk factor for a multitude of cardiovascular diseases, and as such it is responsible for significant morbidity and mortality. Recent advances in vascular biology and mechanics suggest a paradigm shift in hypertension research. It is now clear that focusing on local regulatory activities of the vascular wall that are controlled by mechanotransduction mechanisms promises significantly increased understanding. In this proposal, we will focus on the molecular mechanisms of vascular adaptation in coronary and cerebral arteries and arterioles, and the associated integrated manifestations in vessel morphology and function at the cellular and tissue levels. Toward this end, we have developed a new micro-pig model of renovascular hypertension that allows us to detail the time-course of hemodynamic changes during the development and reversal of the hypertension. Using an externally controllable suprarenal aortic coarctation model, we will delineate between purely mechanical effects and those due to engaging the renin-angiotensin system. This will allow us to explore the hypothesis that the efficacy of pharmacological therapy depends strongly on the target vascular bed and the time that the intervention is initiated during the development of the hypertension. The overall working hypothesis is that hypertension-induced alterations in cell function and matrix biology are largely due to changes in the point-wise multiaxial stress field. Specifically, we hypothesize that altered stresses (intramural and wall shear) induce (1) changes in the local expression of nitric oxide and angiotensin, (2) down-regulation of potassium-sensitive ATP channels and adenosine receptor subtypes, (3) increases in RGD integrin binding sites in the matrix, similar to those in a wound healing response, and (4) spatial and temporal differences in apoptosis and the production of growth factors and proteases. These effects, balanced by a resetting of the barorecptor reflex, shear stress regulation of endothelial activity, and the myogenic response together result in the bed-specific adaptation. These hypotheses will be tested by combining clinical, molecular, cell biological, immunohistochemical, morpho-logical, and biomechanical methods to study coronary and cerebral vessels (n = 5-8 per cohort) at multiple times during the development and reversal of hypertension in a single animal model – although there are many calls in the literature for multidisciplinary attacks on the problem of hypertension, this study will be the first to collect and synthesize such broad data. Indeed, given the vast amount of data, we suggest that combining three recent, separate theoretical developments by members of our team will enable us to develop mathematical models that synthesize the data and provide predictive capability. The latter will enable the exploration of further hypotheses in an efficient manner and guide pharmacologic delivery strategies. Years 1-2 will focus on the time-course of changes due to the development of hypertension whereas years

3-5 will focus on the time-course of changes due to reversing the hypertension either mechanically or via specific pharmacological agents, both as a function of the time during the development of the hypertension and the time that the intervention is initiated.

STATUS OF RESEARCH AND PARTNERSHIP

One new partner was added, but there were no changes in organizational structure. We have continued to make significant progress along two primary lines: continued development of new tools for analysis and new data on histo-biomechanical changes in vascular structure and function due to hypertension. With regard to the former, we extended our theoretical framework of arterial growth and remodeling from an initial 2-D model that addressed single, modest step changes in mechanical loading to models that can address arbitrary step changes in loading; moreover, we implemented these models in finite element codes, which will provide even greater generality. We have also made significant progress in developing new algorithms for automated and semi-automated histological and immunohistological examinations. These algorithms enable objective quantification of gross morphology (e.g., radius: thickness ratios), matrix organization (collagen and elastin density as well as lamellar structure), and cell counting (e.g., proliferating cells as revealed by Ki67 staining). With regard to new information, we have quantified the time-course of histo-mechanical changes in the basilar artery due to hypertension over 8 weeks. Surprisingly, most of the growth and remodeling occurred within the first 2 weeks; this is the first such demonstration in a non-genetically induced model of hypertension. Changes in the circumflex artery appear to occur over a similar time course. Indeed, structural changes in the aorta proximal to the coarctation similarly occur mainly within two weeks. Conversely, we found that functional changes in resistance vessels (i.e., arterioles) do not manifest until much later, typically around 8 weeks in the heart but even later in the brain. This is a potentially significant finding – that large artery growth and remodeling occurs quickly in response to hypertension whereas arteriolar changes appear to occur more slowly. It is not yet clear what causes this dramatic difference. We are in the process of finalizing our analyses of these time-courses, but are also focusing microarray analyses on the 2 week time-point in the aorta. Preliminary results confirm a conversion of smooth muscle cells from a contractile to proliferative phenotype early on, which in turn allows much of the significant remodeling to occur quickly.

ISSUES

We added one new partner in year 4, Professor S. Liu and one of his Ph.D. students, from the Department of Computer Science at Texas A&M University. We had not anticipated the tremendous workload in analyzing many immunohistological sections from multiple sites at multiple time-points during the development of hypertension. They have, and continue to, develop automated and semi-automated algorithms for objective analysis of these images.

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PROJECT TITLE: Multimodal microPET and microMRI Imaging Instrumentation

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT
We propose to combine the best features of Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI) modalities in a single instrument that will simultaneously record data in both imaging modalities. Moreover, we will develop labeled probes that can be detected in both PET and MRI to aid in the interpretation of complex biological processes. This system will be dedicated to the study of small animal model systems at the highest spatial and temporal resolutions attainable. We will build a high resolution, relatively high sensitivity multi-slice μPET scanner integrated within a customized 7T/30cm small animal MR system that will simultaneously record MR and PET images. Through the use of fiber-optic couplings, the μPET system will interfere minimally with the μMR system, enabling high quality μPET and μMRI data to be acquired essentially simultaneously and in near-perfect spatial registration. This system is a natural extension of earlier proof-of-principle systems and a newer prototype animal μPET system now nearing completion. The basic design elements of the system have been tested and demonstrated to work. The combined system we propose adds a number of important features to improve performance and ease of use for in vivo imaging studies. It also incorporates, for the first time, a multi-slice μPET system, with four detector rings simultaneously providing seven imaging planes, spanning an axial field of view of approximately 8mm, with at least 2mm resolution. Simultaneous μPET/μMRI recordings will provide important correlations not available from temporally and spatially separate scans (e.g., BOLD MRI compared with FDG PET). The melded system will provide high resolution anatomical reference systems for μPET studies. The 'in register' μMR images will be used to compute scatter and attenuation in the μPET images and to estimate partial volume errors in the PET scans, thus aiding quantification of the PET signal. This system will open up a number of opportunities not possible with current independent technologies. Among them are:
- Time correlated μPET and MRS studies of drug distributions; cardiac, CNS and tumor cell metabolism.
- Simultaneous fMRI and μPET neuroreceptor brain mapping studies in small animals.
- Validation of new MRI probes using their PET counterparts.
- Dual PET/MRI labels will allow for "zooming-in" the MRI data collection scheme to those regions of the specimen containing the label, as well as providing for precise registration of the PET & MR images.

STATUS OF RESEARCH AND PARTNERSHIP
Initial efforts in the project are aimed at 1) optimizing PET data collection capabilities within a high field MRI magnet; 2) installation of 7T/30cm MR scanner; 3) prototyping potential PET/MRI contrast agents.
Optical Fiber Coupled APDs for the Readout of LSO Crystals in a Simultaneous PET-MRI Scanner.
We are developing a high resolution, high sensitivity multi-ring small-animal PET scanner integrated in a 7T small animal magnetic resonance imaging (MRI) system. There are several approaches to building

such a scanner. The previous prototype built in our lab as a proof of principle had some disadvantages mainly because it was a single ring scanner with limited sensitivity and it involved long optical fibers to couple the scintillator crystals to the photomultiplier tubes (PMTs) located outside the fringe magnetic field. We are now constructing a multi-slice system in which we couple the scintillators elements to avalanche photodiodes (APDs) using short optical fibers. The volume of fibers is in this way greatly reduced and the APDs together with their dedicated printed circuit boards (PCB) and electronics can be placed at a safe distance in their operating range, but outside the MR scanner field of view. This dramatically reduces the chance of radiofrequency interference between the PET and MRI components. To read out the large number of crystals we will use arrays of APDs or position sensitive APDs (PSAPD). Results of the preliminary studies of different configurations of crystals, fibers, charge sensitive preamplifiers and avalanche photodiodes that were considered for this project and present initial results for an APD-based PET detector inside a 7T MRI system are encouraging.

7T 30cm Bruker BioSpec MRI System Installation and Custom Animal Handling Accessories. This 7T/30cm horizontal bore system is designed for research applications using MR in animals in vivo as well as with other small samples. The dimensions of the μPET insert demand the large bore diameter. The relatively high field strength will provide good signal strength in the demanding applications planned in this project. During the first year of this grant we have concentrated on: 1) acquiring the MR system, 2) site preparation, and 3) beginning design of small animal handling/monitoring accessories.

1. The system has been ordered with cost sharing from the Caltech Brain Imaging Center (CBIC) and will be resident in CBIC space in the Broad Biological Sciences Building.
2. Renovations of space are under way, which include adjacent space for animal holding and surgical procedures, a radioisotope lab for manipulation of PET labels, and workstations for data analysis. We expect to take delivery of the MR system in late summer or early fall 2005.
3. Imaging studies using animal models demand that animal motion and experimental conditions be tightly controlled. The 7T/30cm μPET/μMRI system will have the same control hardware and software as 9.4T/30cm CBIC small animal system currently in place, thus we are currently using it to develop animal holders and a physiological monitoring system. Physiological monitoring (ECG, respiration, temperature) is done using a BioPac Systems (Santa Barbara, CA) MP150 with a number of amplifiers and signal transducers. We are currently testing different modifications of the holder and different lead placement for the monitoring transducers.

Prototyping Dual PET/MRI Agents. We have been preparing two main categories of agents for dual-mode imaging:

1. Iron oxide nanoparticle (IONP) based agents conjugated to 64Cu-DOTA
2. Biomolecule based agents consisting of maleylated bovine serum albumin (mal-BSA) conjugated to Gd-DOTA and 64Cu-DOTA. Mal-BSA is specifically taken up by macrophages and we are designing these agents to label early arterial plaques. These are agents being developed for applications in cardiology.

Water soluble iron oxide particles have been synthesized and characterized by TEM and MRI. We are currently exploring methods to introduce amine groups to the nanoparticles. Attachment of 64Cu-DOTA to the coated nanoparticles requires a functional group such as amine. However, initial attempts using literature methods for amination of the coated nanoparticles were unsuccessful. We are therefore deriving new methods to aminate dextran coated iron oxide particles.

Dual labeling of the Mal-BSA contrast agent resulted in a solution that was visible by PET at concentrations down to the nanomolar level. μPET scans of decreasing concentrations of Mal-BSA (DOTA-Gd/64Cu)15 indicated that it is easily observable at 0.0018 μM but that the PET signal is in the noise at 0.00018 μM Mal-BSA (DOTA-Gd/64Cu)15. Studies on uptake by macrophages showed a concentration dependent increase in signal enhancement with observable enhancement at 50 μM. In order to determine specificity of uptake, competition studies were performed. We observed that contrast enhancement is reduced with increasing amounts of competing unlabeled contrast agent; indicative of receptor mediated uptake.

ISSUES

Delays in renovation of space for the 7T/30cm scanner have pushed back its installation date from the originally estimate Spring 2005 to late Summer 2005. The recent availability of APDs and PSAPDs have provided us with the opportunity to redesign significant portions of the μPET instrument to take advantage of these detection devices.

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PROJECT TITLE: Plant Viruses as Platforms for Biomaterials

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT
The overall aims of this project are to explore virus-based protein cage structures as platforms for synthetic modification with direct applications in bioimaging, and targeted drug delivery. The investigators involved in this project have significant experience working in the area of structure–function relationships in viral and related capsid systems. The virus capsid proteins are highly symmetrical supramolecular assemblies and these structures have a number of distinct advantages for their use as precursors for nanomaterials. A) They can be produced with relative ease in large amounts using either their native hosts (plants) or heterologous expression systems (E. coli, P. pastoris, baculovirus). B) An in vitro assembly system has been developed, which allows for disassembly and reassembly of capsid proteins. C) A wide range of genetic mutations can be accommodated by the viral capsids. D) Synthetic methods have been developed for chemically modifying the viral capsids using either endogenous or engineered functional groups. E) Methods and expertise for structure determination are in place to evaluate the structure of modified capsids.

STATUS OF RESEARCH AND PARTNERSHIP
The BRP has continued to function in a highly productive and complimentary manner. The Finn group has significantly expanded the chemistry of cow pea mosaic virus (CPMV) by developing an understanding of anomalous behavior of cysteine reactivities and by developing a new attachment technology employing "click" chemistry. These advances are important for avoiding unwanted side reactions with the virus surface and for improving the efficiency of attaching high value compounds to CPMV. The Montana groups of Young and Douglas have continued to focus on the cow pea chlorotic mottle (CCMV) with excellent progress in the preparation of magnetic resonance imaging agents formed with Gd3+ bound CCMV particles and by the formation of metallic crystals within the particles. They have also succeeded in targeting CCMV and heat shock proteins with monoclonal antibodies to integrins. Zlotnick has demonstrated an exceptional level of control of assembly variants of the CCMV viral subunits through mutagenesis and the control of solvent conditions. The N-terminal residues have a great degree of assembly control and Zlotnick can routinely produce particles with 60 subunits, 120 subunit, 180 subunits and tubes of subunits. These have been structurally characterized in the Johnson group at Scripps and high resolution studies are underway. The variable sized particles will permit a high level of control over the metallic structures made by Douglas and Young. Doerschuk has continually improved the application of the reconstruction algorithms for icosahedral particles. Doerschuk's approach requires no prior knowledge of particle orientation and the reconstructions produced to date are comparable to those performed with conventional programs that require orientation information. The longer-term goal is to apply this approach to asymmetric molecular assemblies with the possibility of clustering particles into

categories based on variations of the assemblies. This will allow the analysis of heterogeneous populations of particles that will be simultaneously clustered and reconstructed. The Lin, Johnson groups at Scripps have focused on cell targeting and killing during the last year. In contrast to the Montana group where chemical attachment has been used for cell targeting moieties and attachment of toxins to the virus, the Scripps group has employed a genetic approach. CPMV particles with a doxorubicin binding polypeptide on the inside and a growth factor receptor binding polypeptide on the outside have been made that will specifically target two types of breast cancer cells and deliver the toxin. Preliminary studies with a mouse model containing an impanted tumor indicate that the particles with the growth factor receptor binding polypeptide do specifically associate with the tumor.

The Montana Groups of Young and Douglas sponsored a meeting entitled Viruses & Protein Cages as Materials in August, 2004 in Bozeman, Montana. Approximately 125 investigators using viruses, heat shock proteins and ferritin for nano medicine and nano materials attended the meeting. This was also an opportunity for our BRP to have its annual meeting. This meeting occurred shortly after the NIBIB investigators meeting in Washington attended by Professor Doerschuk from our BRP. He gave a comprehensive report of the meeting and this affected the directions that we have taken during the last year. Our focus has been much more on cell targeting and drug delivery with a decreased emphasis on conceptual studies of the viruses as reaction surfaces and vessels. Our latest progress report and publications reflect this focus on nano medicine.

ISSUES

The Montana and Scripps groups are now deeply involved in nano medicine research that has been carried out with a variety of cell types to prove concepts of cell targeting and killing with doxorubicin. We are now moving into animal models requiring mouse studies with implanted tumors. The costs for such work exceed the budgets for these groups, as the original budgets were geared more for chemistry. We anticipate that the success demonstrated with these studies will allow a higher level of funding at the time of our renewal application.

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PROJECT TITLE: Digital Mammography With a High-Resolution Flat Panel Imager

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (R01EB2123, formerly NCI R01CA88792)

ABSTRACT
At the present time, there is no consensus on the spatial resolution requirements for digital mammography systems and this has been one of the motivating factors for this research. This bioengineering research partnership with Fairchild Imaging (formerly, Lockheed Martin Fairchild Systems) is aimed at developing and evaluating a new high-resolution flat-panel mammographic imager with variable pixel size (39 and 78 microns). This imager is a 2 x 3 array of large-area CCDs (8 x 8 cm) tiled in a seamless fashion to provide an imaging area of 16 x 24 cm. The image sensor is coupled to a structured CsI:Tl scintillator using non-tapering (1:1) fiberoptics, thereby preserving the spatial resolution without the detrimental loss in the collected signal. Our past experience (not this grant) with the 100-micron pixel GE clinical evaluation prototype in a screening population demonstrated "equivalency" for cancer detection with similar sensitivities. However, it is not clear whether a 100 micron pixel is ideal for detecting all types of microcalcifications and under all conditions. When subtle calcifications are visualized, their edges may not always appear as sharp as that observed with spot film views and this degradation effect may be attributed to the pixel size of the detector. Hence, this investigation was undertaken with the aim to explore the potential for high resolution digital mammography. The research plan calls for development and evaluation of a high resolution experimental prototype digital mammography detector. Moreover, the research involves computational and experimental studies followed by development and comprehensive evaluation of the system through objective and universally accepted metrics, such as the modulation transfer function (MTF) and detective quantum efficiency (DQE). In addition, we will explore perceptual metrics such as contrast-detail (CD) characteristics of the system and compare it to existing clinical mammography systems.

STATUS OF RESEARCH AND PARTNERSHIP
The following major research objectives have been accomplished:

1. _Model refinement:_ A computational model was used to theoretically investigate the potential imaging characteristics of the proposed system. The model reiterates the results of our preliminary computations and provides support for the stated specific hypotheses.
2. _Development of a single module prototype:_ A single module 8 x 8 cm prototype, which operates at 39-micron pixel and is a precursor to the final prototype, has been developed.
3. _Scintillator evaluation:_ Structured CsI:Tl scintillators from two vendors varying in thickness and manufacturing processes such as substrate, coating type and strength, were evaluated. We also

investigated a new pixilated structured CsI:Tl scintillator developed by one manufacturer and preliminary results demonstrate improved imaging properties.

4. *Fiberoptic evaluation:* The appropriate thickness of fiberoptic plate required to provide adequate protection to the CCD has been identified and incorporated into the single module prototype.

5. *Electrical properties of a single module:* The electrical noise properties of the single module 8x8-cm prototype were investigated at room temperature.

6. *Physical imaging properties of single module:* The physical imaging properties of the single module were characterized with a variety of CsI:Tl scintillators.

Research activity in the most recent year

Final Assembly of the large imager: Our partners in this research, Fairchild Imaging, Inc., Milpitas, CA, have delivered the full-size 16 x 24 cm imager and characterization of this device is now under way in our laboratory. We have now confirmed that a small pixel size (39 µm) that enables imaging at high spatial resolution does not result in degradation in the detective quantum efficiency. We consider this an important finding. Although the high spatial resolution is desirable, this comes at the expense of large digital storage space, typically about 50 Mbytes per full-field mammographic image. We have addressed this issue by evaluating the efficacy of digital data compression schemes. Ongoing measurements are concerned with the performance of the large imaging module and its characterization is in progress. This will include a study of contrast detail characteristics.

The partnership has been successful in many respects. It has enabled us to have direct input on various aspects of the design during the entire process rather than just expecting a deliverable. Although the main focus of this research is on digital mammography, this technology can be adapted to other x-ray imaging applications that require high spatial resolution.

ISSUES

The main issues relate to the inherent challenges in certain aspects of the development of the proposed devices. However, we have worked closely with our research partner company, Fairchild Imaging, and we believe we have overcome most of these obstacles. It has been a pleasure and a learning experience to have the opportunity to work closely with our partner company in this research and important results have been derived from this research partnership. Other issues relate to some administrative delays due to the move of our entire research group in 2002 from the University of Massachusetts Medical School to Emory University but all these minor issues have been resolved.

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PROJECT TITLE: Iron Metabolism Alterations in Alzheimer's Disease

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GRANTING NIH INSTITUTE/CENTER: National Institute on Aging

ABSTRACT

Our BRP applies minimally invasive technologies to determine if altered brain iron metabolism in the face of Mild Cognitive Impairment (MCI) represents a significant risk factor for the development of Alzheimer's disease (AD). Goals are development of biomarkers for the diagnosis of AD based on a novel MRI technology (Susceptibility Weighted Imaging – SWI), MRS (Magnetic Resonance Spectroscopy) and iron-related peripheral blood assays (lymphocyte flow cytometry, genomic and proteomic analysis). Sequential MRI and blood studies at six months to a year are correlated with periodic detailed psychometric evaluations on the MCI and control participants accrued over a 30-month period. SWI is validated by studies of mice with an engineered deletion of iron regulatory protein 1 and 2 genes that accumulate excessive brain iron and a neurodegenerative disorder. Assays of microwave fixed mouse brain iron (loosely bound, non-heme, heme, total) are correlated with quantitative SWI signals (phase change measured in radians). 12 specific brain regions from each mouse hemisphere are dissected for analysis.

STATUS OF RESEARCH AND PARTNERSHIP
Human Studies

Participants: To date 79 MCI participants have been enrolled in the study (MCI median age, education, 77, 14; controls, 73.5, 14) after screening 1233 contacts. Attrition over the past 30 months has resulted in a loss of 16 MCI cases secondary to intercurrent illness. The current cohort includes 63 MCI participants, (37 males and 26 females) and 31 controls, (12 males and 19 females). Participants have a yearly SWI, MRS, biannual blood and psychometric evaluations. A 15% annual conversion rate from MCI to AD has been found in our participants.

Special SWI and Spectroscopy: All participants have SWI baseline 3D T1 weighted, fast T2 FLAIR (Fluid Attenuated Inversion Recovery), fast T2 weighted spin echo, and MRS. 63 MCI participants have had sequential studies (32 study two, 11 study three, and 2 study four). In the control group 31 have had study one, 22 study two, and 2 study three. On the basis of studies conducted, cross checking with 5 observers evaluating the same 4 subjects, a very small inter-observer error has been noted in SWI phase measurements. At this point in our study we have found that the variation in phase, an indicator of iron content, follows Gaussian statistics with a standard deviation for the distribution of errors of about 25-30 units. Any deviation of 3 standard deviations or 90 units can be considered a significant variation. Our objective is to correlate neurologic change with abnormalities revealed by phase analysis. Our results so far indicate that it is possible to define a normal distribution of phase for specific brain structures. Our goal is to determine if phase measurements in specific brain regions (correlated to iron content) have the capability of correlating with the severity of neural degeneration. Phase difference measurements are

focusing on the putamen, globus pallidus, caudate nucleus, motor cortex, substantia nigra, and red nucleus. Studies of the hippocampus and frontal basal nuclei in coronal sections are in progress. Sequencing parameters and protocols for SWI and MRS have been standardized. In addition to single voxel spectral acquisitions in the posterior cingulate gyrus – a standard for MCI studies, a multi-voxel chemical shift acquisition technique has been designed with the Siemens Vision platform and is producing excellent spatial quality data. MCI cases, particularly progressive cases, have significantly lower NAA (N-acetylaspartate) (a neuronal marker) than controls. Data acquisition for SWI underwent several trials with different echo times (TEs) and flip angles before settling on a standard protocol with a TE = 40 mins. Serial hippocampal volume determinations are being made and document segmental atrophy in progressive MCI cases.

    Flow Cytometry: Our laboratory has prepared 5 different monoclonal antibodies (Mabs) to the iron degradation domain (IDD) of iron regulatory protein-2 (IRP-2) for flow cytometric studies. Peripheral blood formed elements have been examined for the presence of surface beta amyloid precursor protein, surface transferrin receptor, intracellular ubiquitin, intracellular IRP-2, cytokines, and intracellular beta amyloid protein. The number and sequence of flow cytometric assays are: MCI participants lab-1 64, lab-2 53, lab-3 34, lab-4 21, lab-5 5, lab-6 1 and control participants lab-1 31, lab-2 30, lab-3 22, lab-4 22, lab-5 6, lab-6 0. Cross checking of flow cytometric results in two independent laboratories demonstrated significant inconsistencies for the same specimens and no correlation with clinical course. As a consequence flow cytometric studies were discontinued after 24 months.

    Genomic Studies: In collaboration with Dr. Keith Coon, Translational Genomics Research Institute, annotation of the 63,138 bp of the human IRP-2 gene has been conducted with location of the critical nanopeptide cysteine IDD sequence in exon 5. Oligonucleotide primers have been constructed to all of the 22 IRP-2 exons. DNA sequence information was attempted on 50 formalin fixed AD occipital lobes, 50 control brains. Formalin fixed DNA was too degraded for assay, the study was repeated with fresh frozen brain tissues. DNA sequence information shows no significant polymorphisms in the AD or control IRP-2. Since we have detected no germline mutation in the IRP-2 gene, we hypothesized that observed changes of increased expression of IRP-2 in AD cases could result from post-transcriptional mRNA modifications or splicing mistakes. An examination by PCR (polymerase chain reaction) and real time PCR failed to reveal any splicing errors or expression variants in our controls and MCI cases.

Animal Studies

    "Knockout" Mice, SWI, Iron Assays: The mouse SWI protocol (11.7T) has been applied after initial high morbidity rates of mice in the coil by improved oxygenation and body warming. The artifacts associated with air in the external auditory canals have been eliminated by application of petroleum jelly. Iron assays of microwave fixed milligram quantities of brain have established regional differences of loosely-bound, non-heme, heme, and total iron concentrations. These results are being correlated with SWI signals and extrapolated back to human studies.

ISSUES

1. Participant recruitment: Though we initially met our targeted population for study (75 MCI, 25 control cases) attrition secondary to patient illness or inability to tolerate the MRI studies has been evident. There is also a difficulty in securing NIH-defined ethnicity distributions.
2. SWI in the coronal plane: The hippocampus and subfrontal basal nuclei are early targets for AD pathology and localization. Reformatting the SWI image in the coronal plane to visualize critical areas such as substantia innominata and entorhinal cortex is under current study.
3. Flow cytometry: Cross checking the flow cytometric data for iron metabolism proteins and amyloid on two different flow cytometers in two different laboratories resulted in inconsistent and unreliable data. An extensive analysis revealed no consistent flow cytometric trends during the course of progressive neurologic deterioration.
4. Mouse brain SWI: Significant difficulties in keeping sick animals alive for the 1½ hour study was finally resolved by bringing a neonatologist in for consultation. The animals required body warming, sufficient oxygenation, and controlled anesthesia in order to resolve this problem.

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PROJECT TITLE: Direct Brain Interface Based on Event Detection in ECoG

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT
A number of people with physical disabilities have difficulty performing any physical movement and would benefit from a direct brain interface, an interface that accepts commands directly from the brain. The University of Michigan Direct Brain Interface (UM-DBI) research partnership is a collaboration which includes the Departments of Biomedical Engineering, Electrical Engineering and Computer Science, Physical Medicine and Rehabilitation, Neurology, Surgery and Radiology from the University of Michigan; the Department of Neurology from the Henry Ford Hospital, and the Institute of Biomedical Engineering from the Technical University Graz. These partners propose to address the functional evaluation of a direct brain interface and the optimization of detection methods used in the direct brain interface.

The (time-domain based) template matching detection method developed by the UM-DBI has demonstrated sufficient accuracy in off-line experiments to warrant real-time, on-line implementation and testing with subjects at the University of Michigan and Henry Ford Hospitals who have implanted electrodes for purposes related to epilepsy surgery. (While these subjects are not members of the target user population, the presence of implanted cortical electrodes in these subjects provides a unique opportunity for direct brain interface development). The proposed functional evaluation includes:
1) Development of an on-line, real-time testing system for direct brain interface methods; 2) Examination of the ability of subjects to learn to voluntarily improve event-related potential (ERP) quality and detection performance given appropriate feedback; 3) Determination of the accuracy and speed with which a direct brain interface can be used to perform functional tasks; and 4) Identification of the relationship between the location of electrocorticogram (ECoG) recorded brain events and the activated portion of the brain as observed through functional magnetic resonance imaging.

Improvements in the accuracy by which brain events can be detected will be approached through development and optimization of time-domain based detection methods (performed primarily at UM) and evaluation of the performance of frequency-domain based detection methods on ECoG (performed primarily at Graz). In addition, off-line analysis will be used to 1) Investigate the ability of current detection methods to differentiate between brain activity related to different actions and 2) Determine the increased accuracy of event detection achievable using ECoG versus EEG.

The proposed research is intended to conclusively demonstrate that a direct brain interface based on the detection of human ERPs recorded intracranially can be used for control of functional tasks. While a simple direct brain interface would be a valuable tool for people with severe disabilities, it is intended that an initial interface would also form the foundation for future generations of direct brain interfaces of ever

increasing complexity which would rely on advanced signal processing methods (such as those explored here). Beyond the scope of the proposed work, the results of these studies will form the foundation for clinical testing of the direct brain interface with individuals from the target user populations using subdural electrodes.

STATUS OF RESEARCH AND PARTNERSHIP

Real time system development (University of Michigan). The real-time direct brain interface (DBI) test system is under continuous refinement to allow more flexible testing. A new version of the software that incorporates frequency based features for improved detection accuracy and a quicker response time has been created and tested. Real-time visualization of event-related frequency changes is ready for testing. A method of documenting movements imagined in response to a prompt is ready for final testing.

On-line direct brain interface testing and time-based detection (University of Michigan and Henry Ford Hospital Subcontract). Testing at the Henry Ford Hospital has been delayed while equipment compatibility issues with new clinical equipment are resolved. The use of imagined movements was tested with one subject at the University of Michigan. Modifications to the documentation of imagined movements were made based on these tests. Future experiments will use feedback based on frequency based features.

Correlation of fMRI and ECOG (University of Michigan). A new fMRI data collection protocol utilizing a block design instead of an event-related design was developed and used with two control subjects. This reduces the time required for each task while preserving the power of the experiment, thus allowing us to examine more tasks and perform more extensive anatomical imaging. A systematic study of the difference between this new protocol and the previous protocol is planned. Further quantitative analysis of existing data is planned and will soon be completed.

Development of improved detection methods (University of Michigan and Graz subcontract). The development and evaluation of improved single-channel detection methods continues. A comprehensive analysis of more than 2000 ECoG channels was performed to compare the performance of detection methods developed at the University of Michigan and Graz under different constraints on maximum allowed response time. The quadratic detector based on a two-covariance model (QUAD) and the wavelet (filter bank) detector (WAV) were found to yield similar results with both showing better performance than cross-correlation template matching (CCTM). The QUAD method was therefore selected for on-line implementation because it is algorithmically simpler and easier to realize in the current online framework.

An important component of the QUAD ECoG signal detection method is the procedure for fitting AR spectral models to training data. We have developed a maximum likelihood (ML) method for jointly estimating the AR model parameters and the signal labels from the training data. This new estimation method replaces our previous ad hoc least-squares approach and establishes a firmer theoretical foundation for the training process.

All detection methods currently in use utilize information from only one ECoG channel. However, a previous experiment which combined the detection output of individual channels by simple logical operations showed that utilizing multiple channels can indeed improve the detection performance in some cases. In order to better employ the redundant information in adjacent electrodes, statistical multivariate algorithms such as common-spatial patterns (CSP), Fukunaga-Koontz transform (FKT), independent component analysis (ICA), orthogonal Fisher discriminant (OFD), and relevant component analysis (RCA) were implemented and tested offline. These algorithms were used to construct spatial filters. Feature extraction was either done by wavelets (filter banks) or in the case of CSP, the features were calculated as normalized variance values of specific time windows. The detection results obtained showed that the detection performance can be considerably increased provided that enough training trials are available. For datasets were only 25 or less trials can be used for the calculation of the spatial filters, the improvement was not significant. In order to increase the robustness of the algorithms for the small number of trials that are usually available in our ECoG recordings, model-based covariance estimation and bootstrap techniques are currently being investigated.

ISSUES

Retaining GSRAs. Scheduling subjects.

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PROJECT TITLE: Type I Collagen-Based Nerve Guide for PNS Regeneration

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GRANTING NIH INSTITUTE/CENTER: National Institute of Child Health and Human Development

ABSTRACT

The overall goal of this project is to design, engineer and evaluate in vivo a type I collagen-based nerve guide for peripheral nerve regeneration. The specific aims for the past year focused on the completion of in vivo screening of the design parameters in the rat sciatic nerve model. The final prototype engineered from the optimal design will be evaluated in primates as a potential entubulation repair method for clinical applications. The key design parameters that were investigated included permeability of the nerve guide, axonal growth guiding channels (micro-tubes and filaments), cell growth inductive (bFGF and IGF-II) and cell adhesive (laminin) molecules. These studies were conducted at the University of South Florida. One centimeter defect of rat sciatic nerve was used as the animal model. The in vivo screening studies have been completed and data analyzed. The final prototype for primate studies has been decided in line with the primate training at Duke University. The primate implantation of the final prototype has just been initiated. Overall results of the studies are briefly summarized below.

STATUS OF RESEARCH AND PARTNERSHIP

Prototypes designed and engineered for in vivo screening included: Nerve guides (NG) with 2 permeability properties, high permeability prototype (NGhp) has a MW cut-off at 2×10^6 Daltons and low permeability prototype (NGlp) has a MW cut-off at 19,000 Daltons; Nerve guides with 2 types of guiding channels, micro-tube (NGmt) and filament (NGmf) guiding channels; NGmt with bioactive molecules (bFGF, IGF-II and laminin). The design of various prototypes also took into account the in vivo stability, kink resistance, compressive resistance, and the suture retention strength for implantation by entubulation technique. Nerve autograft (sciatic nerve cut, reversed and rotated by 180 degrees) was used as a control.

Histomorphometrical methods were used as a primary tool to evaluate the outcome of repair. Electrophysiological studies in terms of compound muscle action potential (CMAP) and gastrocnemius muscle weight loss post repair were also conducted for certain design parameters. In the histomorphometrical evaluation, 100% of the myelinated axons (MA) were counted due to a large variation of the axon distribution, particularly for nerve guides containing guiding channels. About 10% of the MA diameters were measured from the mid-sections of the repaired nerves and from the contralateral sciatic nerves.

The salient features of the results are as follows: In the NG group (without guiding channels), there was a trend in favor of NGhp group over NGlp group. There was a continuous increase of the number of MA over time. The total number of MA for NGhp group at 24 weeks approached that of the 12 weeks autograft (AG) group, indicating the NGhp has the potential to repair a 1cm defect. The major weakness of the NG group is its biomechanical insufficiency in vivo (decrease in lumenal area due to distortion of the nerve guide). Thus, the NG alone may not be suitable for longer defect repair.

In the NG group with guiding channels, axonal regeneration is very robust in NGmt group, a four fold increase of MA from 6 to 12 weeks. The tubular morphology was largely preserved due to the presence of micro-tube guiding channels. Micro-tubes were in the process of being resorbed over time. The CMAP data of NGmt supported the histomorphometrical studies (50% of the AG group at 6 weeks). The loss of

gastrocnemius weight for NGmt group was also less than other repair groups, but is somewhat higher than the AG group. However, in the NGmf group, the slow resorption and the aggregation of the filaments had a negative impact on axonal regeneration. Also, the cross sectional area had reduced greatly by 12 weeks.

The AG group had a significantly greater number of MA than the experimental groups at all time points. In the axon counting we included those axons that grew outside the main nerve epineurium sheath (escaped axons). Since the autografts were from the same anatomical site (same diameter, similar structure), this nerve is considered to be the most optimal autograft in comparison to the sural nerve (multiple nerves cabled for size matching) in the clinical practice.

In contrast to studies by other investigators, the results of prototypes incorporating bioactive molecules did not show significant improvement in the axonal growth or the CMAP value over the prototypes without bioactive molecules. Since our goal was to design and develop an off-the-shelf nerve guide for repairing longer gaps, we devoted a substantial amount of effort and resource to evaluate the method of incorporation of bioactive molecules, their activity and stability in storage and upon sterilization by gamma irradiation. The prototypes developed were determined to be suitable for in vivo evaluation. We were puzzled by our results and therefore initiated a second study to investigate the effect of bioactive molecules by using two different methods to incorporate the bioactive molecules. One method was the same as that developed by us and the other was the incorporation of bioactive molecules prior to prototype implantation to simulate those studies by other investigators. Again, we did not observe any significant improvement by the incorporation of bioactive molecules by both methods of incorporation.

The results of the screening studies have led us to the following conclusion. To fulfill the function as an alternative to the nerve autograft and to bridge longer nerve defects (>2cm), the nerve guide must be biocompatible, biomechanically competent, be able to guide axonal regeneration across a longer gap, and be kink resistant for repairing the nerve across a joint. The nerve guide with micro-tube guiding channels is the best candidate at this time. We have just initiated a primate study to repair the median nerve with a longer gap using NGmt. The primate studies are summarized below.

The primate study at Duke University is divided into two groups. One group is to repair a 2cm median nerve gap with NGmt. Sural nerve autograft is used as controls. Behavioral methods will be applied as the key parameter to evaluate the return of function. After several iterations, a final grip and shear task has been designed to enable accurate assessment of fine motor recovery following median nerve transection and repair. The training of primates is now at the point suitable for conducting behavioral studies. In addition, electrophysiological studies in terms of CMAP and CSAP (compound sensory action potential) will be monitored over time to corroborate the behavioral studies. The animals will be sacrificed at the end of the study and the repaired nerves will be analyzed by histomorphometrical methods.

In the second group, a 5cm median nerve gap will be repaired by NGmt to test the limit of a long gap repair. Only electrophysiological and histomorphometrical methods will be applied to this group. The primate surgery has just been initiated.

In summary, progress has been made in regard to the specific aims set forth in the proposal. Thus far, the program has proceeded according to schedule.

ISSUES
None.

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PROJECT TITLE: Live Microscopy and Cytometry in Vascular Biology

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GRANTING NIH INSTITUTE/CENTER: National Eye Institute

ABSTRACT

The broad aims of our program are to develop new technologies for visualizing, tracking, and quantifying cells in living animals, and to apply these technologies to problems related to the vascular biology in the eye. The program brings together investigators from biomedical, biophysical, and bioengineering disciplines, using advanced instrumentation to study animal models of diseases such as angiogenesis, diabetic retinopathy, and sickle cell retinopathy. We are developing a flexible and transportable in vivo confocal microscope/scanning laser ophthalmoscope (SLO) (Project I.1) with capability for high-speed confocal imaging at up to 200 frames/second in order to track fast moving cells in the retinal vasculature (Project I.2). In parallel, we are developing an SLO with wavefront sensing and correction technology to achieve diffraction-limited single cell resolution in the mouse retina (Project I.3). Finally, we are developing an in vivo flow cytometer to detect and count individual fluorescently labeled cells in the circulation (Project I.4).

The imaging systems will be used to study the vasculature in development and pathology in mice (D'Amore, project II.1). Molecular probes for vascular permeability and for vascular endothelial cells (EC) will be developed (Hamblin, project II.2) to study cellular mechanisms responsible for endothelial dysfunction in diabetic retinopathy (Bursell, project II.3) and the vaso-occlusive processes in sickle cell retinopathy in vivo (Lutty, project II.4).

STATUS OF RESEARCH AND PARTNERSHIP

We have built a new video rate laser scanning platform with an interchangeable front end that can work as a confocal microscope or a scanning laser ophthalmoscope. Compared to our previous system, the new scanner has a more compact footprint, simplified electronic circuitry, and an updated computer interface to handle more demanding image acquisition and processing tasks. In addition to multiple fluorescence excitation/detection channels, the new system has improved polarization optics for imaging backscattered light that is useful for identifying tissue morphologic features where fluorescence signals are acquired. Due to aberrations inherent in the optics of the mouse eye, we have not yet achieved diffraction-limited resolution when imaging the mouse retina. Ongoing effort will use adaptive optics (AO) to compensate for the wavefront error of the mouse eye. Even without AO, however, the image quality is high enough to visualize cell trafficking in the mouse eye in vivo.

With Dr. Jerry Lutty's group at Johns Hopkins, we are conducting experiments to develop a better understanding of the cellular interactions that result in microvascular occulsions in sickle cell disease.. We have developed methods to label and visualize the passage of sickle red blood cells (RBC) and white blood cells (WBC) through the retinal vasculature of live mice. In sickle transgenic animals (developed

by Nagel et al at Albert Einstein University), we observed repeated cell adhesion and release in certain areas of the retinal vasculature that were not seen in beta thallasemic control mice. Notably, the ssRBC adhered primarily in capillaries, while the WBC were adhering and rolling primarily in large veins.

With Dr. Allen Clermont and Dr. Sven Bursell at Joslin Diabetes Center, we are investigating methods of in vivo imaging of the retina using markers associated with retinal endothelial cell dysfunction and retinal inflammatory processes. Using proteomics approach, the Joslin group has recently identified the intracellular enzyme carbonic anhydrase 1 (Ca-1) as a vitreous proteome specific to the proliferative diabetic retinopathy (PDR). Intravitreal injection of Ca-1 at concentrations observed in human vitreous from patients with PDR, markedly increases retinal vascular permeability to fluorescein, Evan's blue dye, and high molecular weight dextran. The magnitude of the effect was equivalent to that of the potent permeability factor VEGF. Acetazolamide blocked the permeability effect of human PDR vitreous when injected into the rat eye, indicating that carbonic anhydrase activity accounts for a substantial portion of the vitreous effects on retinal permeability in PDR. These results demonstrate a novel action of extracellular carbonic anhydrase as a physiological activator of vascular permeability. The activity of Ca-1 on RVP was both additive and independent of VEGF. Intravitravitreal injection of Ca-1 induced a 3-fold increase in leukocyte adhesion in the retina compared with eyes that received the saline vehicle injection at 48 hrs post injection. This effect of Ca-1 was blocked by co-injection with 10 _M acetazolamide. In addition, Ca-1 injection into the vitreous a diabetic rodent model is associated with the development of short-term retinal edema as shown by high resolution OCT. The Ca-1 results provide a molecular link with diabetes-induced retinal vascular permeability and intraretinal edema and point to the selective Ca-1 inhibitors as potential therapeutic agents for the treatment of excessive retinal vascular leakage and retinal edema associated with conditions such as PDR, macular edema, and macular degeneration.

In vivo flow cytometry is a new technology developed with the support of the BRP program to detect and quantify circulating cells in live animals without the need to draw blood samples. We can monitor circulating cells continuously in live animals over time spans from a few hours to many weeks. We are using this technology to monitor T cell circulation kinetics, and to detect the migration of antigen presenting cells from the eye into the systemic circulation. Using markers for apoptotic cells, we have successfully detected cancer cells undergoing apoptosis in the circulation, and we have measured the kinetics of the apoptotic cells as they are cleared from the circulation (Wei et al, Molecular Imaging, 2005, in press). Measurement of circulating apoptotic cells may prove to be useful as a monitor for early response to therapies that induce apoptotic in tumor cells or endothelial cell (i.e. anti-angiogenic therapy). Similarly, b measuring the depletion kinetics of circulating leukemic cells, we can assess the effects of agents that prevent the engraftment of the leukemic cells to their target tissue (Sipkins et al, Nature et al 435: 969-973). In summary, in vivo flow cytometry can be used to screen the effects of

ISSUES

There are major personnel changes this year involving the relocation of two of our partners to other institutions. Dr. Steve Burns is moving from SERI to Indiana this summer, but Dr. Burns will continue to be available for consultation, and Dr. Robert Webb will take over as the PI of the AO project at SERI. Dr. Webb is a long-time collaborator of both Dr. Lin and Dr. Burns, and has participated in this BRP from the beginning, so we have not experienced any problems with the transition. We will also continue to work closely with Dr. Tom Bifano's group at Boston University and will have access to their latest micromachined deformable mirror technology. Another partner, Dr. Alexei Bogdanov, recently moved from MGH to University of Massachusetts an Amherst. Fortunately we are starting a new collaboration with Professor Moungi Bawendi at MIT to develop new quantum dots for in vivo imaging, so our probe development effort will not be affected.

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PROJECT TITLE: Multi-modality Biomedical Imaging

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ABSTRACT
Our project is based on the belief that information provided by nuclear medicine images may sometimes be ambiguous and that a better way to use imaging technology would be to obtain images from multiple modalities, and spatially co-register them with sufficient accuracy that values from different images could be numerically correlated with each other. We are applying this approach to understand PET images of tumor hypoxia, with the aim of using oxygen probes, histological information and NMR, to show how reliable different PET tracers of hypoxia really are.

STATUS OF RESEARCH AND PARTNERSHIP

a. *Tumor hypoxia imaging by PET; comparison with pO2 probe data*

We have imaged tumor hypoxia in rodent models with the tracers 124I-IAZG, 18F-FMISO and 64Cu-ATSM, using a microPET (Concorde Microsystem) with spatial resolution of 2 - 3 mm. In initial studies, we compared 124I-IAZG and 18F-FMISO microPET images in MCa breast tumor- and FSa-II fibrosarcoma-bearing mice. Both 18F-FMISO and 124I-IAZG show high uptake in large tumors with high hypoxic fraction, but not in small, low HF tumors. Subsequently, we compared 18F-FMISO and 64Cu-ATSM by serial microPET imaging in R3327-AT Dunning rat prostate tumor- and FaDu xenograft-bearing rats. In the rat hosts, tumors of up to 3 cm diameter can be studied, facilitating the evaluation of heterogeneity (regions with different pO2 levels) within the tumor.

We reported last year that in the R3327-AT prostate tumor model, late 64Cu-ATSM images (16 hr p.i.) exhibited different spatial distribution from early 64Cu-ATSM images (2 hr p.i.). 18F-FMISO distribution was similar to that of late 64Cu-ATSM images. Subsequently we repeated this experiment in a FaDu tumor model. Our data showed that the distributions of early and late 64Cu-ATSM images were similar, and no different from that of 18F-FMISO Thus, we conclude that the kinetics of 64Cu-ATSM may be cell type dependent, and could affect the distribution of the tracers at different time p.i.

Direct pO2 measurement in tumors was also performed using the Oxylite probe system. Based on the measured pO2 levels, a pO2 profile was generated using the Excel graphics software. Approximate correspondence was observed between the regions of low pO2 and the regions of high 18F-FMISO uptake. In other words, there is qualitative agreement between the tumor hypoxia distributions in this tumor as measured by the Oxylite probe and imaged by 18F-FMISO-microPET.

b. *Visualizing tumor hypoxia: a comparison of hypoxia controlled gene expression and exogenous markers*

In an effort to relate the molecular mechanisms associated with tumor hypoxia with non-invasive hypoxia imaging, we have adopted the method of imaging the expression of reporter genes. Specifically we have stably transduced R3327-AT cells with the expression vector pHRE-TKeGFP in which the TKeGFP is controlled by the hypoxia-inducible promoter. Stable clones were isolated, designated as R3327-AT(HRE-TKeGFP). R3327-AT(HRE-TKeGFP) cells were transplanted into nude rats to form tumors. On each of the rats, two other tumors were also transplanted, one from

parental R3327-AT cells, and the other from R3327-AT cells stably transduced with the vector pCMV-TKeGFP (designated R3327-AT(CMV-TKeGFP)). When the tumors reached an average diameter of ~ 2 cm, Ci 124I-FIAU through□Ci 18F-FMISO and 200 □the rats were co-injected with 2,000 the tail vein. Imaging with the microPET was performed at 3 and 24 hr post p.i. Because of the difference in injected activities and decay characteristics (~1 positron per decay for 18F, and per 4 decays for 124I), and the slower incorporation of 124I-FIAU relative to that of 18F-FMISO, the signal from the 3 hr p.i. image of the R3327-AT(HRE-TKeGFP) tumor was due mostly to the trapping of 18F-FMISO in the hypoxic regions of the tumor. In contrast, at 24 hr p.i. (> 12 half-lives of 18F), more than 99.98% of 18F has decayed, and the image would be due entirely to the trapped 124I-FIAU in TK-expressing cells (half-life of 124I ~4.2 d).

The 3 hr p.i. image shows 18F-FMISO uptake in each of the three tumors. In contrast, the 24 hr p.i. image (indicating only 124I-FIAU activity), shows no 124I-FIAU trapping in the parental R3327-AT tumor, abundant radiotracer retention in the R3327-AT(CMV-TKeGFP) tumor constitutively expressing TKeGFP, and some 124I-FIAU incorporation in the R3327-AT(HRE-TKeGFP) tumor. These data provide support to our hypothesis that the TKeGFP gene, controlled by a hypoxia-inducible promoter, and stably transduced and integrated in the R3327-AT(HRE-TKeGFP) cells, was induced in vivo in the R3327-AT(HRE-TKeGFP) tumor, in hypoxic regions that were also visualized by microPET - 18F-FMISO imaging.

These data were subsequently confirmed in experiments in which a) the intratumoral distributions of 18F-FMISO and 124I-FIAU were compared using digital autoradiography and b) the distributions of 124FIAU (as determined by DAR) and pimonidazole (as determined by IHC) were compared. The good spatial co-registration between the distribution of 124I-FIAU and pimonidazole and between 124I-FIAU and 18F-FMISO underscores the potential of the R3327-AT (HRE-tkeGFP) tumor model as a possible reference standard for tumor hypoxia imaging.

c. *Comparative studies using two types of pO2 probes*

In laboratory studies with rodent tumors, we have compared the use of the OxyLite and Hypoximeter pO2 measurement systems. In a recent experiment, we performed pO2 measurements with both systems on the same R3327-AT tumors implanted on the flank of nude mice. Tumors with volume ranging from 0.36 cc to 0.93 cc were assessed with the two probes in parallel trajectories (4 trajectories per probe in an interleaved manner). 52-84 measurement points were obtained per animal per probe.

The data show variations in pO2 values in each tumor, with a similar range for both systems. A more detailed analysis of these preliminary data also support the findings of other laboratories that both systems yield similar results in terms of pO2 distribution within tumors.

d. *Registration of images from microPET, NMR and tumor sections*

Using the (previously described) multimodality imaging stereotactic template system, we performed a study in which a male nude Copenhagen rat was anesthesized, immobilized with the form-fitting mold, and firmly attached to a specially-designed positioning platform that is common to the microPET and NMR scanners. The R3327-AT tumor bearing limb was positioned directly below the template. The animal was first imaged with NMR, then with microPET / 18F-FMISO, and then sacrificed and tumor sections prepared for fluorescent microscopy of Hoechst (injected 1 min before sacrifice) and pimonidazole (injected 2 h before sacrifice). For NMR and microPET imaging, the cap of the template was fitted with gadolinium markers and 68Ge sources respectively. The NMR and microPET data sets were then registered based on the images of the gadolinium markers and the 68Ge sources.

Thus, we are improving our capability of integrating and comparing images from multimodality non-invasive and invasive approaches, and the tremendous potential of such imaging methods to provide scientific insight relative to tumor hypoxia.

ISSUES
None.

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PROJECT TITLE: BION Treatment of Neuromuscular Dysfunction

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT
In theory, a wide range of sensory and motor dysfunctions can be treated by electrical stimulation to evoke patterns of neural activity similar to those that underlie normal function. In practice, however, such stimulation has typically required relatively expensive and large devices implanted by a surgeon or skin surface stimulation applied by a trained therapist. We have developed a platform technology that can deliver precisely metered stimulation pulses to an arbitrary number of nerve and muscle sites to treat a wide range of clinical problems. BIONs (registered trademark; BIONic Neurons) are a new class of chronically implantable stimulators. They are single channel, wireless electronic microstimulators (16mm long x 2 mm in diameter) that can be injected in or near muscles and nerves. Each BION receives power and digital command data from a single, externally worn transmission coil to produce stimulation pulses with controlled current (0-30mA) and duration (4-512 microseconds). BIONs have been demonstrated to produce stable thresholds at their deployment sites and have been shown to be safe and effective for stimulating muscles in animals. Results from ongoing small cohort clinical trials have shown them to be effective in preventing and reversing shoulder subluxation and increasing knee function in patients with knee osteoarthritis. Under this BRP, we are designing and building BION 1 implants and accessory components for their testing, programming and control in patients. We are applying this technology to a range of clinical problems following stroke to determine safety and efficacy and to further understand the mechanisms underlying neuromuscular pathology and treatment:

- Shoulder subluxation
- Flexion contractures of the wrist and fingers
- Retraining of voluntary hand function

We are also extending the BION technology to increase power efficiency and range, improve controller design and portability, and incorporate sensors of posture and motion as well as sensor and back-telemetry capabilities for functional electrical stimulation (BION2S and BION2). As these enhancements become available, we will expand the clinical applications to provide more complete rehabilitation of multi-joint dysfunctions that commonly occur in stroke, explore other clinical applications and incorporate advanced control algorithms for closed-loop control to provide functional reanimation of paralyzed limbs.

STATUS OF RESEARCH AND PARTNERSHIP
Three BION clinical trials sponsored by the A.E. Mann Institute for Biomedical Engineering at the University of Southern California (AMI-USC) are funded by this grant. They include two trials at Rancho Los Amigos National Rehabilitation Center: "BION Treatment of Post Stroke Subluxation (FDA IDE:

G010068; Rancho IRB protocol #1879, 05/16/05; and, USC IRB protocol #019038,10/23/04) and "Comparison of Exercise Programs for Wrist and/or Finger Flexor Tone and Contracture Management" (FDA IDE:G030147; Rancho IRB # 1950; 02/28/05; and USC IRB protocol #03B016, 06/02/05). The FDA-IDE and IRB applications for the "BION-induced Training post-Stroke to Enhance Recovery of Hand Function" clinical trials have been approved (FDA-IDE: G040143, 11/01/04; USC IRB: 04C015, 12/09/04) and will begin after we have verified and validated various changes to the BION design and manufacturing process that are now underway. Subject recruitment for all AMI-USC sponsored BION clinical trials, including the three trials funded by this grant, was voluntarily and temporarily halted on March 18, 2005 due to similar device malfunctions in four subjects. In every case, the device was broken at the tantalum end. A manufacturing review was held within 24 hrs of inspection of radiologic confirmation of the break in the first subject (March 10, 2005). None of the broken devices have migrated from the site of implantation, though one of the broken pieces of one device had rotated by 180 degrees. No symptoms other than loss of function have been reported and there are no plans to remove the devices surgically unless this is indicated by a medical problem. Changes in the position and state of the device will continue to be monitored in these subjects.

The failure analysis has produced a clear indication of the mechanism and mode of failure, which has been replicated on the bench. Briefly, the problem affects 4 of a total of 79 BIONs that we have implanted in various trials since 1999 and appears to be related to the recent use of strong tetanic contractions in relatively muscular patients where the BIONs are oriented crosswise to the muscle fascicles. If the tantalum electrode is entrapped in connective tissue outside the muscle, it is possible for substantial bending forces to be applied to the thin tantalum stem where it enters the glass capsule. Even a few degrees of bend cause ductile flow of the tantalum and a stress riser at the entrance into the glass that initiates a crack in the glass bead. With repeated stress, this crack propagates through the entire capsule, causing the gross disruption seen on the radiographs.

Research and development are continuing on 1) a new BION insertion tool and automatic localization based on M-wave recordings, 2) a second generation integrated circuit with sensing and back telemetry capabilities, 3) redesign of external coil drivers for power and communication, and 4) a new BION controller that will support wireless programming and real-time control algorithms for functional electrical stimulation (FES).

ISSUES

We have identified three different strategies to eliminate the problem and are well into testing the simplest and most desirable option. This involves welding the glass bead that forms the seal to both the tantalum stem and the tantalum electrode body, eliminating the bendable neck that appears to be the source of the problem. If validation test results continue to be positive, we could be in a position to restart the clinical trials as early as the end of this summer. We are also using this interval to test a new integrated circuit, which is designed to increase compliance voltage while lowering power consumption of the BION implants. This will make it easier for clinicians to implant and for patients to use, so we will probably incorporate and validate that change, as well. Clinical studies will resume as soon as design changes are validated and approvals have been obtained from the FDA and the IRBs. We expect to complete the studies as originally designed.

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PROJECT TITLE: Shape Memory Polymer Devices for Treating Stroke

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT
We propose to develop interventional devices for treating stroke victims that currently have no therapeutic alternatives (~400,000/yr in the USA). The development and testing of two complementary devices is proposed: a mechanical clot extraction system and a neurovascular stent. The clot extraction system will address the current clinical need for an acute ischemic stroke treatment and the stent will address the chronic problem of stenosis and/or restenosis of the neurovasculature. Both of these devices utilize photomechanical micro-actuators based on laser-activated shape memory polymer (SMP).

SMP is a material that will have a significant impact on clinical medicine. SMP is a relatively new material that is similar to shape memory metals in its ability to actuate from an initial deformed shape into a second, pre-determined shape. Shape memory metals are currently very popular in medicine as a material for making vascular stents. SMP has advantages over shape memory metals for certain applications, including cost, higher recoverable strain levels, ease of manufacturing, better flexibility in navigating tortuous paths, and great versatility in fabricating extremely small, highly complex actuators. Potential applications of SMP include stents, stent release mechanisms, embolic coil release mechanisms, thrombus extraction devices, and many others.

The underlying hypothesis of this research is that mechanical devices can be used to treat stroke victims where there is currently no clinical alternative. There are five known private companies that are currently pursuing this hypothesis for the acute ischemic device and an unknown but presumed large number of companies pursuing neurovascular stents. Members of the current proposal team originally developed one of the technologies that is in FDA trials for treating ischemic stroke, photo-acoustic emulsification of the thrombus. However, in our opinion, none of the current devices under FDA trials is as promising or as straightforward as the devices proposed. Further, we believe that the technology developed and published from the proposed studies will lead to many other medical applications that are far beyond the scope of one proposal and one team of investigators. The proposed research is a unique combination of biomaterials, lasers and optics, immunology/biocompatibility and clinical interventional neuroradiology.

The long-term goal of this research is to deliver clinical prototype devices that can begin FDA clinical trials.

STATUS OF RESEARCH AND PARTNERSHIP
We are on track for all fourth year research goals described in the proposal. The key aims of the fourth year are animal trials with the second-generation devices. The second-generation stents have been

fabricated and preliminary animal trials are scheduled in the fall of 2005. A working neurovascular stent delivery system has been successfully demonstrated. The development of both the clot extraction and polymer stent devices is progressing well. All of the project components including device engineering, materials development and characterization, biocompatibility studies and interventional studies are under way. The ischemic stroke devices have been integrated into standard guide wire (coil) and catheter (basket) devices and there is a strong possibility that they could lead to commercial devices after the animal trials. The in vitro biocompatibility studies show that SMP is essentially equivalent to Teflon for cytokine and platelet activation. The in vivo biocompatibility studies have shown some inflammation response but we are currently redesigning the study to determine if the response is from the surgery or materials. Functional animal studies of the acute ischemic stroke device are underway. Beyond the original scope of the proposal, we had, and took, the opportunity to undertake preliminary studies in three distinct areas: shape memory foams, synthesize new shape memory polymer materials (two different materials, acrylic and a different urethane from the Mitsubishi materials), and magnetic field heating/actuation of the materials. These spin-off studies have concluded are being written up as publications. We have also received seed funding for both the foam (NIH SBIR with Sierra Interventions, Melodie Metzger is the PI, Duncan Maitland is the LLNL co-I) and new materials (LLNL internal peer review award, Tom Wilson is the PI). By the end of the fourth year we expect to have 20+ patent applications filed and 15 peer-reviewed publications. Finally, we are in licensing discussions with two major medical device companies and two venture backed start up companies.

ISSUES
None.

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PROJECT TITLE: Morphological and Functional Musculo-Skeletal Imaging

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GRANTING NIH INSTITUTE/CENTER: National Institute on Aging

ABSTRACT
Participants from the University of California San Francisco (UCSF), Lawrence Berkeley National Laboratories (LBNL) and Industry (Focus Imaging, Exponent Failure Analysis, General Electric) propose to form a Bioengineering Research Partnership (BRP) focused on the systematic study of the morphology and function of the musculoskeletal system in disease and health. In addition, resources from existing research relationships with General Electric Medical Systems, SUN computers and IBM will be combined and utilized to rapidly evaluate and disseminate the developments of the BRP. The aim of this consortium is to improve medical care through bioengineering developments, and to facilitate close interactions between bioengineers, computer scientists, clinical investigators, basic scientists and corporate partners. This effort will expedite the development of clinically-relevant quantitative imaging tools and propel the technical advances from the laboratories into the operating rooms and clinics. We hypothesize that high resolution, fast magnetic resonance imaging techniques and positron emission tomography, combined with quantitative image analysis, processing and visualization, can provide new insights and clinically viable and relevant methods for objective evaluation of disorders of the musculo-skeletal system. The long-term objective of this partnership is to understand the link between morphology, function, biochemical changes and clinical symptoms in the musculo-skeletal system. An immediate objective is to develop, implement and optimize novel non-invasive imaging methods (magnetic resonance imaging: MRI and positron emission tomography: PET) that will allow us to depict the musculo-skeletal system, quantitate morphology, function, provide unique 3D visualization and graphical representations of function and morphology, as well as correlate these with biochemistry and clinical status. This research partnership is aimed at quantitating early degenerative changes in two clinical areas of emphasis: the knee and the spine. The first phase of the partnership will be technique development, followed by testing, and ultimately evaluation in case control studies in symptomatic patient populations. The specific goals are: (i) to develop quantitative morphological and functional markers for degenerative diseases of the spine, (ii) to develop quantitative morphological and functional markers for the degenerative changes in the knee and osteoarthritis.

STATUS OF RESEARCH AND PARTNERSHIP
The immediate objective is to develop, implement and optimize novel non-invasive imaging methods (magnetic resonance imaging: MR and positron emission tomography: PET) to depict the musculo-skeletal system, quantitate morphology and function, and provide unique 3D visualization and graphical representation of function and morphology. The first phase of this research partnership is aimed at

quantitating early degenerative changes in two clinical areas of emphasis that have major societal impact: the knee and the spine. The Positron Emission Tomography and Nuclear medicine developments were expensive and did not provide significant results. As a result we redirected the BRP to include other imaging modalities. The BRP has expanded the imaging scope to include Fourier transform Infra-red Imaging, and High resolution Magic Angle Spinning (HRMAS). In addition the plans of extending combined Magnetic Resonance, Computed Tomography to imaging the spine and knee have progressed considerably, and the proecess of execution has been fine tuned. The BRP has led to several accepted publications, abstracts and presentations at meeting. The goals of the BRP of integrating scientists from UCSF, clinicians and scientists at UC Berkeley and Lawrence Berkeley National Laboratories have proceeded as anticipated, leading to an integration of engineering and biomedical applications. In due course we have had to make changes to our operating procedures, and modified the methods of collaboration between sites at the administrative level. Confusion with different campuses have been mitigated through joint appointments. The significance of the partnership lies in the cohesive efforts of bioengineers, computer scientists, and clinicians in developing quantitative musculoskeletal imaging methodologies. The large number of publications and also the proposed RO1 applications in the next year make this is a fruitful and productive effort.

ISSUES

The competing renewal for the project has been submitted. The challenge now is the successful renewal of the BRP so the efforts that have been made thus far are not halted.

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PROJECT TITLE: Processing of Materials for Improved Biocompatibility

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT

Purpose: The purpose this research partnership is to obtain fundamental understanding of a novel, low temperature process for sterilizing and cleaning biomaterials and biomedical devices. The new process is intended to improve biocompatibility. Cleaning, particulate removal, and sterilization are currently separate steps that are crucial to the viability of medical devices. As medical implants grow more complex and as new biomaterials are developed for advanced applications, there is a crucial need to develop new techniques and processes that can clean and sterilize a wide variety of materials and devices at moderate to low temperatures, without introducing potential contamination, and without damaging the surfaces or otherwise compromising the biocompatibility or the functionality of the device. This project will provide the necessary science and engineering basis for evaluating a new low temperature process for cleaning and sterilizing based on liquid or highly compressed carbon dioxide (CO_2), and for determining if the technology is more effective, less expensive, and more benign than technology based on steam, ethylene oxide, hydrogen peroxide, or radiation. The research is broadly applicable to the manufacture of biomaterials, implants, and prostheses.

Methods: The first thrust is to determine conditions under which supercritical CO_2-based fluids sterilize microbial spores; both USC and Clemson University have conducted these experiments on both immobilized spores ("spore strips") and free spores, using standard cell culture methods for quantifying the kill rate. The second thrust is to determine the mechanisms of spore killing. Scanning and transmission electron microscopy, proteomics (2D gel electrophoresis + MALDI-TOF analysis of extracted proteins), fluorescent staining and optical imaging, and analysis of the release of dipicolinic acid are among the assays used for mechanistic studies. The third thrust is to examine the mechanical integrity and biocompatibility of various medical polymers after treatment with CO_2-based fluids. Mechanical integrity (e.g., wear resistance, tensile strength) is quantified using standard ASTM methods, while biocompatibility is assayed using in vitro and in vivo (rat model) methods.

Results: Much of the current work is focused on optimizing sterilization and understanding the mechanism by which the process occurs. The secondary focus is the effectiveness of cleaning (removing contaminants from surfaces), whether the contaminant is manufacturing debris (oils, particulates) or biological (e.g. bacterial debris). We have clearly confirmed that exposing spores to CO_2 alone, or to CO_2 + water, is not sufficient to achieve 6-log reduction of spores. However, parts per million (ppm) quantities of 30% aqueous H_2O_2 solution in CO_2 does result in 6-log reduction of B. atrophaeus, G. stearothermophilus, and B. pumilis spores. We have also initiated work on B. anthracis. Spores treated with CO_2-based mixtures release small quantities of dipicolinic acid, and we have tentatively identified small proteins that appear to be from the interior of B. anthracis. TEM imaging shows considerable disruption of the glycoprotein exosporium. The mechanism of spore death appears to be CO_2-facilited

disruption of the exosporium, causing oxidation or disruption of the interior membrane followed by trace release of DPA and proteins from the spore interior. Work continues on the mechanisms. Both titanium-based orthopaedic implant materials and expanded Teflon ™ vascular implant materials remain biocompatible after CO2 treatment, as evidenced by rat subcutaneous implant models and a variety of in vitro assays. Medical grade crystalline plastics such as UHMWPE show good tolerance (mechanical integrity) after CO2 processing, although there is some evidence that UHMWPE may tend to delaminate after processing. However, amorphous plastics such as natural rubber will swell significantly in CO2; therefore, medical devices fabricated from such plastics may not be compatible with CO2 sterilization. Visual observation shows that CO2 + hydrogen peroxide are not effective in removing bacterial debris from surfaces contaminated with S. aureus, although the treatment does kill S. aureus. Long, narrow lumens inoculated with S. aureus are not completely sterilized by CO2; thus, in future work more attention must be given to facilitating contact and mass transfer in such geometries.

Conclusions: Further results and details will appear in the following articles:

- Sterilization using high-pressure carbon dioxide-A review. Zhang, J.; Davis, T. A.; Matthews, M. A.; Drews, M. J.; LaBerge, M.; and An, Y. H. Journal of Supercritical Fluids, 2005, accepted.
- Sterilizing Bacillus pumilus spores using supercritical carbon dioxide. Zhang, J.; Burrows, S.; Matthews, M. A.; Drews, M. J.; LaBerge, M.; and An, Y. H. Journal of Microbiological Methods, 2005, accepted.
- Identification of marker proteins for B. anthracis using MALDI-TOF MS and ion trap MS-MS after direct extraction of electrophoretic separation. Stump, M.J.; Black, G.; Fox, A.; Fox, K.F.; Turick, C.E.; and Matthews, M.A. J. Separation Science, accepted, May 11 2005.
- Mechanisms of sterilization of B. atrophaeus spores using supercritical carbon dioxide. Zhang, J., Matthews, M.A.; Gleason, C.; Waller, L.; Fox, A.; Fox, K.; Drews, M.; Laberge, M.; and An, Y.H. In preparation/invited paper for a special issue of the Journal of Supercritical Fluids in honor of Professor Aydin Akgerman. (June 2005).
- Effects of sterilization on implant mechanical property and biocompatibility—A concise review. An, Y.H.; Drews, M.D.; LaBerge, M.; and Matthews, M.A. Submitted to Int. J. Artificial Organs). In review, May 2005.
- Polymeric biomaterials: Compatibility of medical-grade polymers with dense CO2. Jimenez, A.; Thompson, G.L.; Matthews, M.A.; Crocker, K.; Lyons, J.S.; and Trapotsis, A.. Submitted to J. Biomedical Materials Res. B., December 2004. In Revision, May 2005.

STATUS OF RESEARCH AND PARTNERSHIP

The current Bioengineering Research Partnership is concluding its third year of initial operation. A no-cost extension to May 2006 has been granted for the current project. Three refereed papers have been accepted for publication, and five more are submitted or in preparation. In addition, eleven presentations at professional meetings have been made. The competitive renewal application for this project was submitted in May 2005, and will be reviewed in November 2005. A new collaborator, Dr. Alvin Fox, University of South Carolina School of Medicine, has been added to the team to provide additional expertise in microbiology of infectious bacteria. Industrial interest remains strong; three companies have agreed to participate in the activities of the renewal grant, and two invention disclosures have been submitted. Two students undertook brief industrial internships in the summer of 2004.

ISSUES
No input.

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PROJECT TITLE: Partnership for MR Spectroscopic Imaging Data Processing

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT

MR Spectroscopic Imaging (MRSI) offers considerable potential as a diagnostic imaging technique; however, its use has been limited by complex data processing and analysis requirements. Optimally, both processing and analysis require integration of a priori spectral and spatial information, including MRI-derived tissue segmentation, morphological analysis, metabolite MR parameters, and knowledge of normal tissue metabolite distributions. This Partnership will: 1) develop an integrated set of processing tools that simplify implementation of MRSI for routine diagnostic imaging studies; and 2) map proton-MR-observed brain metabolite distributions in normal subjects over whole-brain, and evaluate changes as a function of acquisition and subject variables.

This effort combines development of MRSI and MRI data processing software under 5 projects located at 4 institutions. This initial development will be followed by data acquisition at multiple collaborative sites. Software will be developed for automated MRSI processing, tissue segmentation, brain region mapping, statistical analysis, and clinical presentation. Results from MRSI and MRI studies will be converted to standardized intensity units and transformed into normalized spatial coordinates, enabling the data to be pooled to form a database of MR-measured human metabolite values. This information will then be used to enhance statistical analysis of individual MRSI studies and map metabolite distributions in normal human brain. The resultant technical developments will be evaluated for diagnostic neuroimaging applications, with an emphasis on 1H MRSI of cancer, epilepsy, and neurodegenerative disease.

STATUS OF RESEARCH AND PARTNERSHIP

In this third year most of the major program modules have been sufficiently developed to enable integration with the other modules, to form a single set of processing tools. These processing functions can be predefined and set up in a pipelined processing environment to enable all MRSI and MRI processing to be carried out in a fully automated manner. Integration of modules written in different programming languages, while making use of a common data-management system using Java, has proved successful, though additional effort is required to coordinate error handling.

A manually-labeled brain atlas has been created, based on the simulated BrainWeb MRI data (Montreal Neurological Institute). Methods are in place to apply spatial transformations to the processed metabolite images into this standardized image space, with a variable target resolution from 1 to 4 mm.

Data acquisition for development of the normal-subjects database has commenced at 1.5 Tesla, which has led to the first production of whole-brain maps of mean cerebral concentrations of N-Acetylaspartate, creatine, and Choline. Additional data sets continue to be acquired to improve the resultant quality and to provide sufficient statistical power to determine standard deviations across a group of subjects, and to enable evaluation of changes in metabolite distributions as a function of subject parameters, notably age.

With the increased availability of 3T MRI systems, additional data acquisitions will also be carried out for this field strength.

The partnership remains unchanged. Perhaps the greatest challenge is maintaining communication between geographically separated sites. PI and individual programmer meetings have been held and conference calls have been used extensively to coordinate development efforts.

ISSUES

The continued evolution of MR methods and instrumentation require frequent reassessment of the functionality to be provided by this project. For example, with the increasing use of phased-array acquisition methods additional support for this data acquisition method has been added. In addition, an alternative spectral intensity normalization method is under development and modifications to the tissue segmentation methods will be required.

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PROJECT TITLE: Leukocyte Trafficking From Flowing Blood Tissue (HL-70537)

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT
This Bioengineering Research Partnership proposal combines expertise from the Biomedical Engineering Departments at Georgia Institute of Technology/Emory University and Rice University with the Section of Leukocyte Biology from Baylor College of Medicine to examine the detailed sequential processes involved in movement of leukocytes from flowing blood to migration in tissues. A systems approach is presented, with the goal of identifying the crucial molecular mechanisms involved at each step and then integration of the steps as would occur in vivo. Both in vitro and in vivo (principally mice) models will be employed - the former to test specific molecular hypotheses and the latter to ensure that mechanisms identified in vitro are of importance in the actual in vivo setting. Three specific aims are proposed: Specific Aim 1: The study of the effects of fluid shear and the interactions of leukocytes and endothelial cells on adherent leukocytes. This aim will use cone-plate viscometry and parallel plate flow systems to investigate the influence of shear on secretory functions and phenotypic changes in adherent neutrophils. Specific Aim 2: The study of the interactions of leukocytes and endothelial cells under shear conditions and the effects on vascular permeability. This aim will use both in vitro and in vivo experimental models to investigate the sites of neutrophil adhesion and transmigration, and changes in endothelial and vascular permeability. Specific Aim 3: The study of the mechanisms of leukocyte migration through extracellular matrix, and the phepotypic changes induced by the processes required for transendothelial migration. This aim will utilize a synthetic mimetic of extracellular matrix to investigate the contributions of proteolysis, adhesion and haptotaxis in vitro, and intravital microscopy to investigate migration through extracellular matrix in vivo. Basic bioengineering expertise is crucial for the success of each Specific Aim and for the integration of aims - involving aspects of biomechanics, transport phenomena, complex biological systems, cellular engineering and biomaterials. We believe the results of these interdisciplinary studies, combining quantitative bioengineering models, novel biomaterials, basic leukocyte biology and fundamental vascular biology will lead to significant advances in our understanding of leukocyte trafficking, with important implications in both normal physiology and various pathological states.

STATUS OF RESEARCH AND PARTNERSHIP
Our BRP employs tissue engineering technologies to evaluate neutrophil interactions with the extracellular matrix (ECM)-mimetic peptides in two and three dimensional systems. We have used a polyethylene glycol (PEG) diacrylate derivative to form a hydrogel that provides a biologically inert surface. Covalently attaching bioactive moieties into the hydrogel has made it bioactive. The goal is to define the mechanisms by which these moieties influence the interactions of neutrophils with this bioactive hydrogel, and thus understand the likely effects of similar ligands in the ECM. These findings will then be tested in vivo employing a murine model. The current experiments analyze the interactions of isolated human neutrophils with PEG hydrogels modified with Arg-Gly-Asp-Ser (RGDS), known ligand

for some β1 and β3 integrins, and Thr-Mer-Lys-Ile-Ile-Pro-Phe-Asn-Arg-Leu-Thr-Ile-Gly-Gly (TMKIIPFNRLTIGG), ligand for Mac-1, a β2 integrin. Our results demonstrate that neutrophils, independent of chemotactic stimulation, show little ability to adhere to unmodified PEG hydrogels. However, cell adhesion and spreading are robust on peptide-modified hydrogels. Incorporating distinct bioactive peptides, either alone or in combination, has enabled recognition of differential functions of αvβ3, β1 and β2 integrins on neutrophil adhesion and spreading. Combined interactions result in activity that differs markedly from that seen with either integrin independently engaged (see paper in Tissue Engineering,10:1775-1786,2004). This model allows investigation of specific ligand-induced leukocyte functions and the development of engineered matrices with defined bioactive properties. Studies on motility in both two and three dimensions (quantitated with video microscopy) are now well underway and a manuscript on this has been submitted for publication. We are in the second year of our partnership and progress has been quite good. We meet monthly via videoconference for 2 hours. This allows detailed discussion of data obtained (2 way video including use of powerpoint presentations), analysis of potential problems and the planning of future experiments. I travel to Houston every three months to directly interact with my Co-PI's and research staff at Baylor College of Medicine and Rice University.

ISSUES

Challenges include maintaining good information flow – as each laboratory has areas of special expertise that all in the partnership need to be able to use to accomplish our multidisciplinary goals.We have found that an Annual Retreat is very helpful. Our last Annual retreat was held on February 3-5, 2005 in Houston. Our next one will be in February of 2006. We are optimistic that progress will continue to be rapid.

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PROJECT TITLE: Nano Arrays for Real Time Probing Within Living Cells

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT
The overall goal of this research is to exploit the development of rigid, vertically aligned, carbon
nanofiber (VACNF) arrays to provide nanoscale probes for mapping and influencing intra- and
extracellular molecular events in and around living cells. VACNF are synthetic structures that self-
assemble in a vertical orientation with respect to a planar substrate and that dimensionally span across
multiple length scales, featuring nanoscale tip radii and lengths up to tens of microns. They may be
deterministically synthesized on a variety of substrates (silicon, quartz, glass), with a high level of control
over many parameters including length, tip diameter, aspect ratio, physical location on the substrate, and
surface chemistry. In this effort, nanofiber probing arrays are being fabricated into devices that feature
individually-addressable, nanofiber based electrochemical electrodes where only the extreme nanoscale
tip of the fiber is electrochemically active. The nanofiber serves both to elevate the electroanalytical
measurement volume above the planar substrate (i.e. within and around cells as opposed to in-between the
substrate and cellular matrix) and to electrically bridge between the nanoscale dimensions of the fiber tip
and the microscale dimensions of the electrical interconnects of the substrate. Additionally, in this
research effort, nanofiber-device fabrication approaches are structured around incorporation of
microfluidic cell and analyte handling strategies, thereby providing architectures that will enable future
high-throughput screening applications, such as clinical diagnostics of cell and tissue specimens and
pharmaceutical exploration and discovery.

Research tasks are focused around several aims: fabrication of robust, nanofiber-based probing
architectures; electroanalytical characterization of nanofiber-based electrodes against benchmark analytes
as well as biologically-relevant species; investigation of cell/fiber interfacing schemes; and measurement
of electrochemically-active species in and around cellular matrices.

STATUS OF RESEARCH AND PARTNERSHIP
In the third and final year of this effort, we have expanded the diversity of nanofiber based
electroanalytical probing devices to begin to tailor them to specific end-user applications. Embodiments
include individually addressable nanofiber elements at 2, 4, 6, 8, and 10 micron pitch providing multiple
discrete elements per single cell; bulk addressed nanofiber arrays with individual elements at 5, 20, and
80 micron pitch providing millions of parallel elements per 10 mm2 regions of tissue; and individually
addressed 40 element linear arrays of nanofibers at 20 micron pitch for interfacing across a region of
excised peripheral nerve bundle. We are also in the final production stage of an individually addressable,
thin film transistor based nanofiber probing array featuring 400 elements over a 1 mm2 footprint
(50 micron pitch). Samples of these devices are now being used in several research laboratories outside of

the initial BRP including those of David Schmidtke (Oklahoma University) for development of electrochemical glucose monitoring, Alan J Bard (University of Texas Austin) for study of catalysis at nanoscale tips, P Khalsa (SUNY Stonybrook) for peripheral nerve interfacing, and Marc Dichter (University of Pennsylvania) for interfacing to hippocampal neurons.

In our laboratories, long term extracellular interfacing of electrochemically-active nanofiber arrays with cell cultures has been performed for periods up to 8 weeks using excitable cell matrices including quail neuroretina (QNR), rat thoracic aorta (A10), NGF-differentiated rat pheochromocytoma (PC12), and day 18 rat hippocampal neurons (E18). In these experiments, cells were cultured directly onto the microfabricated electrode devices and individually-addressed nanofiber electrodes were used to both stimulate and record excitable activity and released electroanalytes at discrete times during extended cell culture. Experiments are underway to now directly interface these arrays into preformed tissue, including excised peripheral nerve bundles and tissue analogs of cells cultured on flexible elastomeric substrates.

Long term intracellular interfacing of passive nanofiber arrays (not electrically addressed) has also been demonstrated in a variety of cell types including Chinese hamster ovary (CHO), PC12, MVLN, and J774a.1 for the purpose of manipulating the intracellular domain via biochemically functional molecules tethered to the penetrant nanofiber scaffold. These latter experiments have included the delivery and expression of free and nanofiber-tethered DNA using both nanofibers, as well as silicon-dioxide-based 'nanopipes'. Nanopipes are akin to traditional glass capillaries, being parallel arrays of nanodimensioned silica tubes templated from nanofiber precursors. Our demonstrations provide basis of application of these pipes as a new approach to parallel patchclamping of large numbers of cells.

In the final months of this effort, we are focusing on merging these efforts to provide intracellular integration of actively addressed nanofibers for probing of electroactive species within viable cells. Follow on competing continuation applications focus on real-time transduction of gene expression within viable cells and electroactuation of the functionality of species tethered to intracellular nanofiber arrays.

ISSUES
None.

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PROJECT TITLE: Force Transmission in the Central Nervous System

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Institute of Child Health and Human Development

ABSTRACT

The partnership is designed around three central areas: cellular mechanics, molecular measurement technologies, and neurotherapeutics. These areas are integrated and applied to the study of traumatic brain injury injury (TBI). Our main objective is to use expertise from these three areas to identify the significant molecular and mechanical factors that contribute to cell death following TBI, and to use these data in developing treatments for reduce cell death in TBI. We focus on the molecular changes that lead to apoptotic and necrotic cell death in the cortex, using a combination of in vivo and in vitro models in identifying the appropriate targets for intervening to repair neurons in the brain after injury. Having identified potential therapeutic targets with this approach, we then examine if these targets are viable treatment strategies for TBI.

STATUS OF RESEARCH AND PARTNERSHIP

In the area of cellular mechanics, our long term goals were to (a) develop cellular-based models of brain tissue, and (b) identify the significant mechanosensitive receptors activated following mechanical injury. Our cell-based models would be used to build constitutive relationships for brain tissue that more accurately reflect the cellular architecture. In comparison, our work identifying the mechanosensitive receptors would be important to identify which downstream signaling cascades are activated following TBI.

We completed the goal of developing cellular based models of brain tissue, using measurements of cellular structures in organotypic brain slice culture models to develop and validate composite based models of brain tissue. From these analyses, we showed that the strains experienced by individual cells within even a small brain region vary significantly. By itself, this heterogeneity at the cellular scale can explain some of the heterogeneity observed in TBI. We used these cell-based models to develop a corresponding constitutive relationship for brain tissue that matches constitutive properties measured by other investigators in past studies.

We identified the NMDA receptor as the most significant mechanosensitive receptor activated following in vitro mechanical injury to both dissociated neuronal cultures and organotypic brain slice cultures. We used a recombinant receptor approach to determine the source of this mechanosensitivity, and have determined that the receptor sensitivity is a direct result of the receptor linkage to the cytoskeleton. Synaptically localized NMDA receptors have the highest mechanosensitivity, while extrasynaptic receptors are only activated at higher levels of mechanical injury. We studied how this regional mechanosensitivity affects the activation of downstream signaling cascades, and identified that JNK activation can occur uniquely through activation of the synaptic NMDA receptors. With the well accepted role of JNK activation and neuronal apoptosis, these data suggest that the direct activation of NMDA receptor following stretch can lead directly to a cell death pathway. We are now examining the

timing of this activation, expecting that the sustained activation of JNK following mechanical injury will lead to conditions that cause cell death.

For molecular measurement technologies, work in the past year examined more completely the subset of genes that are activated following stimulation of the mechanosensitive NMDA receptor. Data from microarray studies, independently verified by QRT-PCR, showed that NMDA receptor stimulation will activate genes involved in both the JNK and ERK signaling pathway, as well as genes involved in synaptic remodeling. We discovered novel genes previously not associated with the JNK pathway, and have shown that some of the genes are directly activated by synaptic NMDAr stimulation. Analysis for two of the most highly differentially expressed genes (RAC1 and GNAS) demonstrated a correlation between the observed differential gene expression after traumatic brain injury and corresponding protein translation. By observing which unique proteins are upregulated following synaptic NMDA receptor stimulation, we may identify new downstream targets for altering the activation of downstream MPAK following traumatic mechanical injury.

Based on the information from the cellular mechanics and molecular measurement areas of the partnership, the neurotherapeutics group initiated two interrelated treatment studies. Given the mechanosensitivity of the synaptically localized mechanoreceptors and the ability of this receptor subpopulation to stimulate JNK activation (see above), we tested whether the inhibition of JNK or, alternatively, the selective deletion of JNK activity using transgenics would alter the neurobehavioral and histological impairments following experimental TBI. Our second treatment study tested whether the direct inhibition of synaptic NMDA receptors (provided by Y. Auberson, Novartis) would alter outcome after experimental TBI. Inhibiting JNK activation using JNK3 -/- mice or treatment with a JNK inhibitory peptide resulted in a significant improvement in neurobehavioral outcome after TBI. In addition, reducing JNK activity following TBI also reduced cell loss and lesion volume after TBI. As expected, inhibiting synaptic NMDAr activation caused a reduction in the activation of JNK, Studies are nearly complete to evaluate if this antagonist based reduction in JNK also causes an improvement in neurobehavioral impairments or a reduction in cell loss following experimental TBI.

ISSUES

Based on the results from some of the treatment studies, we are now completing longer term studies to assess if the reduction in JNK activity shows a sustained improvement following TBI. In addition, we will test if the neuroprotective effect can be sustained with a delayed administration of either the JNK inhibitory peptide or the receptor antagonist. We are also screening additional potential compounds for potential neuroprotection in both our in vitro and in vivo models of TBI. We will report on the results from this study at next year's meeting.

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PROJECT TITLE: Sensing and Processing for Directional Hearing Aids

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Institute on Deafness and Other Communication Disorders

ABSTRACT

The aim of this effort is to develop revolutionary technology for hearing aids that will lead to a marked improvement in the ability of the hearing impaired to understand speech in noisy environments. Our focus is on improving the technology of acoustic sensing and processing of signals so as to minimize the influence of unwanted sounds. We will accomplish this by a highly coordinated team effort to ensure that the design parameters of each feature of the system are mutually optimized and are compatible. This effort may be viewed as having three closely interrelated areas of technology development: novel directional microphones, novel optical electronic readout, and novel signal processing. These three areas are briefly described in the following:

Novel Directional Microphone Diaphragm Design: A highly innovative microphone diaphragm concept will be developed that will provide the following advantages over existing approaches: approximately 10 dBA lower thermal noise so it is usable in both quiet and noisy environments, high acoustic sensitivity that will facilitate electronic readout, and high robustness so that the design can be manufactured at low cost through bulk microfabrication techniques.

Novel Optical Electronic Readout: The achievement of radical improvements in microphone performance listed above will in large part be made possible by the incorporation of new technology for converting the diaphragm motion into an electronic signal. We propose to adapt optical technology for detecting the diaphragm motion that will enable the removal of key design constraints associated with capacitive sensing, the "standard" approach in small microphones. The removal of the design constraints associated with capacitive sensing will permit a revolution in microphone designs and will enable the achievement of greater sensitivity and lower noise.

Novel Signal Processing: The revolutionary microphone technology to be developed in this effort will also enable the development of signal processing schemes that enhance the system's ability to reject unwanted noises. By tailoring the signal processing algorithm to the novel microphone technology used here, we will be able to develop a prototype system that achieves 2 to 5 dB improvement in the reduction of unwanted sounds beyond what is possible with existing hearing aid technology.

STATUS OF RESEARCH AND PARTNERSHIP

The administrative elements of this partnership are well established and working well. The team has bi-weekly meetings (teleconferences) in which they review and discuss the current project status, twice-yearly meetings over a two-day period where they meet to discuss progress made and plans for the future, and monthly progress reports which are submitted to their NIH/NIDCD Program Manager.

During the second year of this project the team has designed and fabricated several optimized designs for the microphone diaphragms that show enormous potential for simplifying the fabrication process and increased sensitivity and performance; successfully fabricated a microphone diaphragm and backplate; designed and evaluated prototype electronics to test the electrical performance of the microphones; completed the integration of the optical sensor electronics and optical design for testing purposes; recorded acoustic signals using the fabricated diaphragms on a hand-held optical sensing set-up; completed several acoustic scene analysis experiments in a variety of environments with a variety of microphone arrays and acquired large datasets for evaluation; completed experiments that confirm the ability of the algorithm/sensor system to achieve significant noise reduction from speech sources.

ISSUES

One long-standing challenge has been the fabrication of the differential microphone diaphragms with backplates that serve the dual purpose of providing protection to the diaphragm from dust and providing a means of electrostatic actuation and/or sensing of the diaphragm motion. We have now successfully fabricated backplates made with low-stress silicon nitride and a thin layer of evaporated chrome and gold. This process produced "complete" microphone arrays with relatively flat backplates and high yield. At present, these microphones are undergoing acoustic testing and analysis. Preliminary results look promising. A signal processing challenge was cleared up after much analysis and processing of data indicated reduced directivity of the three-omni microphone array in a free-field environment. An important discovery was made that provided evidence that the reduction was due to a multitude of factors, each contributing incrementally to the shortcoming in directivity.

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PROJECT TITLE: Development of a Bidirectional Brain Machine Interface

PARTNERS' NAMES AND AFFILIATIONS:
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(Northwestern University); Ranulfo Romo (National Autonomous University of Mexico)

GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke

ABSTRACT
Recent technological advances have made it possible to move a robotic device in real time, using signals obtained directly from the brain. This field of Brain Machine Interface (BMI) has the means to provide movement for paralyzed patients, communication for locked-in patients, and a better understanding of the brain for all of society. In order to control movement effectively, the brain must be able to activate muscles appropriately, and monitor the evolving movement quickly and precisely. Existing BMIs, while remarkable, do each of these tasks in poor imitation of the intact nervous system. Our proposed work addresses these limitations by developing a bidirectional interface that produces movement in a more natural way, and provides feedback about the movement by direct, electrical stimulation of the brain.

Our partnership includes members at Northwestern Univ (NU), Univ of Chicago (UC), Univ of Mass, Amherst (UMass), and the autonomous Univ of Mexico (UNAM). Partners have advanced degrees in a range of biological science, computer science, physics, mathematics, and engineering disciplines. Miller (NU) will coordinate the partnership. He has extensive experience with a wide range of recording, stimulation and behavioral protocols in behaving monkeys. Hatsopoulos (UC) is at the forefront of the field of multi-electrode recordings. He was a leading member of the first group to demonstrate visually guided BMI control by a primate. Barto (U Mass) has done pioneering research in neural networks, machine learning and stochastic optimization. Fagg (UMass) is an authority in the control of reaching and grasping robots that learn to interact with the environment. Together they will develop the decoders of activity from the brain used to cause movement. Romo (UNAM), is a world leader in studies of the perceptual and decision making processes induced by electrical stimulation of the brain. Solla (NU), is an expert in neural networks and information theory. With Romo, she will develop optimal routines to encode information in stimulus trains to provide feedback to the brain. Mussa-Ivaldi (NU) will focus on the overall design and evaluation of the interfaces. He created the first ever bidirectional interface between neural tissue and a robotic device.

STATUS OF RESEARCH AND PARTNERSHIP
Our project has been underway only two months, so progress has necessarily been limited. We have continued to hold monthly video conference meetings of the entire partnership, which have been quite successful, involving as many as 15 participants among all the groups. We have also established an interactive web site to facilitate data and figure sharing during smaller phone conferences. Since the time of the application, the following progress has been made on aims 1-3.

1. _To determine the effectiveness of novel decoders of neuronal signals for the control of a virtual arm._
   The primary effort in this aim has been the use of various model learning techniques to predict arm

motion from cell activity. Arm motion is described in a variety of coordinate systems, including: Cartesian position, velocity, acceleration; and joint position, velocity, acceleration, and torque. To date, decoders that predict intrinsic variables (in particular, joint acceleration and torque) perform better than other decoders (especially Cartesian position). We are investigating the use of both linear (e.g. PCA) and nonlinear techniques to reduce the dimensionality of the cell activity representation. This approach has reduced some of our problems with over-fitting (for the gradient descent and pseudo-inverse methods), and has improved the performance of techniques that rely on distance computations in these high-dimensional spaces (specifically, k-nearest neighbors and locally-weighted regression). We are working to incorporate spinal feedback into the decoding process, and to develop simple structured models of muscle/spinal interaction as a component of the decoding process.

2. *To develop a unidirectional afferent BMI to encode virtual arm state for movement guidance.* Initial experiments have been undertaken in a single monkey to determine the feasibility of stimulation within the proprioceptive region (area 3a) of the somatosensory cortex as a source of proprioceptive feedback. A monkey was trained to move to one of two targets when it was illuminated. Initially this visual cue was paired with 3a stimulation, the leftward and rightward targets paired with low and frequency stimulation respectively. After several sessions, the monkey was able to detect the electrical stimulus, discriminate between the two frequencies, and use the stimulus to initiate its movement to the correct target in the absence of the visual cue. We are currently working to extend this simple target designation paradigm to one in which actual limb state is conveyed by the stimulation.

3. *To evaluate the performance of a unidirectional, efferent BMI with proprioceptive feedback.* We have accomplished two important components of aim 3. First, we have successfully implemented a real-time, closed-loop decoder of Cartesian hand position using a simple, linear filter decoder. By recording single and multi-unit activity from up to 55 electrodes implanted in the arm area of primary motor cortex, we have successfully generated predictions of hand position updated every 50 ms which specify the cursor position seen by the monkey. The monkey was able to move the cursor through a sequence of randomly positioned targets with a mean time-to-target of 1.7 s using brain control as compared to a mean time-to-target of 1 s using hand control. Second, we have demonstrated that we can drive the robotic exoskeleton (KINARM) via our experimental control computer to arbitrary positions using a linear elastic force with damping. We are now in a position to drive the robotic exoskeleton via brain control to positions dictated by the decoder and thus provide the monkey with the opportunity to use its own arm as a proprioceptive channel for positional feedback.

ISSUES
No input.

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PROJECT TITLE: New Approach for the Treatment of Asthma

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT

Asthma is an often debilitating disease characterized by dyspnea, wheezing, coughing, respiratory distress, and sometimes death. Subjects with asthma typically have hyperresponsive and often chronically inflamed airways. Chronic asthma is also characterized by extensive airway remodeling, with a thickening of airway walls, increased mucus glands and goblet cells, increased vascularization, and most importantly, a hypertrophy of airway smooth muscle. Although there are a multitude of different possible triggers, an acute asthmatic attack is always characterized by contraction of the smooth muscle in the airway wall. Much of asthma research in recent years has focused on an understanding the immunologic factors that often lead to asthmatic attacks. Nevertheless, whether the initial cause results from an allergen, an irritant, psychological stress, or other neural activation, the cascade always ends with airway muscle constriction. It would thus seem most sensible to treat asthma by minimizing the ability of this smooth muscle to contract, and ongoing studies in this Biomedical Research Partnership will allow optimization of a biomedical device to do just that.

STATUS OF RESEARCH AND PARTNERSHIP

This BRP involves the design, construction, and testing of a biomedical device that can limit the ability of the airway smooth muscle to narrow the airways. The work involves a close working partnership between the physiologic laboratories and expertise at the Johns Hopkins University and Asthmatx, Inc., that is providing the mechanical and bioengineering skills needed for product development. The overall long term objective of this research is to develop and evaluate an innovative and potential clinical treatment for asthma.

Results in the fourth year of this partnership have continued with the experimental testing in the canine model, and publishing this work in the literature. There were 2 new publications and 2 more in review. Work on the first 2 specific aims in the original proposal have now been nearly completed, although this work on the design and modification to the existing thermal probe has actually led to new insights into how to make even better improvements. This effort during this past year has been greatly aided by the computational fluid dynamic modeling work which has now been submitted for publication. This model has also clearly shown that the differences in electrical and thermal conductivity between the airway wall and the parenchyma significantly affect the resulting transient temperature distribution in the airway wall and the parenchyma. Ongoing work will include modeling the effects of anatomical heterogeneities, determination of tissue properties, and in vivo confirmatory experiments. This information will provide essential insight into design issues that will be pursued in the competitive renewal application.

Experimental work at Johns Hopkins in the past year was concerned with two experiments, that of how the thermoplast treatment affects the distensibility of the airways, and whether the treatment can affect airway closure. In the first, we now clearly show that bronchial thermoplasty treatment significantly alters the ability of airways to dilate with lung inflation. The treated airways are larger at any given level of lung inflation, this is true in both relaxed and contracted airways. This is an important finding that may impact on how airways respond to deep inspiration in asthmatic subjects. This work has now been

published. In another new study, which has been submitted, we have exaimend the effectiveness of treatment on the ability of airways to close completely. We had previously developed a system to challenge airways sufficiently with agonist to make them close, and have used this technology to study the effectiveness of Alair treatment in preventing this. Our results show substantial reduction in the ability of airways to completely close. Given the current high level of clinical interest in the role of airway closure in the observed hetereogeneity of ventilation in asthmatics, this knowledge may have important ramifications if bronchial thermoplasty reaches the clinical stage.

ISSUES
Not applicable.

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PROJECT TITLE: Novel X-Ray Technology for Degenerative Joint Disease

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GRANTING NIH INSTITUTE/CENTER: National Institute of Arthritis and Musculoskeletal and Skin Diseases

ABSTRACT

The non-invasive detection of early or mid-stage pathological cartilage changes, prior to any bone changes, in degenerative joint disease is of importance so that behavior modification, disease modifying agents, and other treatment regimes may be undertaken in a timely manner. The current gold standard of diagnosis of degenerative joint disease is conventional radiography, a method that addresses only joint space narrowing as a result of cartilage loss and bone changes such as sclerosis and osteophytosis. By this stage, the joint is most likely committed to a pathological progression. Furthermore, at least one study suggests that conventional radiographs are unreliable for evaluating cartilage loss in patients with early OA since, in most cases, joint space narrowing is secondary to meniscal extrusion rather than thinning of cartilage. Diffraction Enhanced Imaging (DEI) is a novel radiographic method, still in experimental stages, that introduces selectivity for the angular deviation of x-rays traversing the subject. It uses a collimated x-ray beam produced by a perfect crystal monochromator. When this beam passes through the subject, a matching analyzer placed between the subject and the detector converts the angular changes in the beam into intensity changes, giving rise to enhanced contrast. Our experiments are carried out at the National Synchrotron Source at Brookhaven National Laboratory, but the DEI technology is not intrinsically tied to a synchrotron and efforts are underway to translate the technique to a compact source of x-rays. We have found that cartilage lesions display as contrast heterogeneities on DEI images. Because the refraction (half points on the rocking curve) images highlight edges, it was here that we are best able to identify lesion outlines and, therefore, determine the severity of a lesion, and whether or not it involves just the articular surface or involves deeper layers as well. Since DEI is a transmission radiographic technique it depicts actual morphology, i.e., the shapes observed on the images are representative of those of the specimen. For instance, surface fibrillation is seen as a roughening of the specimen surface on a DEI image. If the fibrillation is deeper into the tissue, it appears as very small contrast heterogeneities (darker regions as compared to surrounding lighter area) within the depth of the cartilage. A fissure is seen as a contrast heterogeneity in its shape. If a lesion only interrupts a portion of the thickness of the cartilage specimen and does not compromise the full width running parallel to the x-ray beam, the cartilage appears intact in its height but a contrast heterogeneity will be present in the shape as the lesion itself. All lesions can be followed in their entirety through the depth of the cartilage. Using human observer data, we found high correlation between two DEI image readers and the actual grossly observable grade of cartilage degeneration on human tali (on the order of .8) and with a high inter-observer reliability. Several lesions proved to be a challenge in identification if studied only in the anterior-posterior view. For instance, it was

occasionally difficult to decipher a Grade 3 erosion from a Grade 4 erosion if much of the cartilage loss was parallel to the x-ray beam (or in the anterior to posterior direction on the talus), but did not cover the full width (medial to lateral) of the image of the cartilage. This was, of course, a result of our current two-dimensional system, which is the reason we took images in the medial/lateral plane as well. This solution would be more difficult, however, for joints such as an intact ankle because of the interference of the bony malleoli of the tibia and fibula in the path of the beam when the ankle is imaged in the medial to lateral position. Although we have previously shown that cartilage can still be visualized on DEI images even when superimposed by bone, we have yet to determine if this is depth or thickness dependent. For a joint such as the knee that can be imaged in several radiographically-friendly positions, we believe the planar mode will not be a significant problem.

STATUS OF RESEARCH AND PARTNERSHIP

With the combined efforts of biochemists and anatomists from Rush University Medical Center and physicists and engineers from the Illinois Institute of Technology, the Diffraction Enhanced Imaging (DEI) Partnership has developed to further advance the non-invasive imaging of articular cartilage and other soft tissues of synovial joints. DEI is a novel radiographic method, still in experimental stages, that introduces selectivity for the angular deviation of x-rays traversing the subject. It uses a collimated x-ray beam produced by a perfect crystal monochromator. When this beam passes through the subject, a matching analyzer placed between the subject and the detector converts the angular changes in the beam into intensity changes, giving rise to enhanced contrast. We have found that cartilage lesions display as contrast heterogeneities on DEI images, thus allowing the qualitative identification of lesions. A human observer study for the validation of DEI for such imaging has shown the technology to be both accurate and reliable. This work was carried out on human tali dissected from organ donors so that the greatest possible cartilage contrast provided by DEI could be explored. Once established, the technique can be applied to a human observer study on intact human synovial joints, including knee and ankle. Thus, our next set of experiments will include the DEI imaging of intact human knee joints from donors of the Gift of Hope Organ and Tissue Donor Network of Illinois. Because DEI allows the simultaneous imaging of both cartilage and bone, the relationship of these tissues in early osteoarthritic development may be observed.

Carrying the DEI technology one step further is Multiple Image Radiography (MIR) which calculates the angle spectrum at each pixel and extracts images based on x-ray small angle scattering (on the order of 1 micro-radian) thus depicting fine textural features of tissues (<50 microns). The final images can be seen as "absorption", "refraction" and "scatter" images, thus depicting these properties, primarily. We have recently explored CT-MIR in which the object is placed on a rotation stage whose rotation axis is parallel to the y-axis. At each tomographic view angle, the MIR method is implemented and the three images (absorption, refraction, scatter) are computed by extremely complicated equations. By considering the sets of measurements acquired at all view angles, and by using a 2D filtered backprojection algorithm, volumetric images can be reconstructed. Our CT-MIR of a human talar head clearly shows the compact and trabecular bone of the talus as well as the surrounding articular cartilage. Although our current imaging time are relatively slow (hours), this issue will be addressed in the coming sets of experiments.

ISSUES

Contrast is not the only parameter of significance in producing DEI images of excellent quality, as the optimization of resolution is a further complication. Our imaging experiments are carried out at the National Synchrotron Light Source at Brookhaven National Laboratory where we are equipped with a Fuji Medical Systems, (model BAS2500). The resolution of the image obtained is limited by image plate resolution which is approximately 50 microns. Additionally, we have a digital detector of approximately 50 micron resolution. These detection systems allow us to visualize cartilage and bone lesions, but do not provide information on the collagen fibrillar level, as we have previously achieved at the Synchrotron in Trieste, Italy. To this aim we have submitted an NIH shared instrumentation grant (SIG) for the acquisition of two new detectors, provided resolutions of 5 and 10 microns. Concerning the issue of lengthy imaging times, we are currently reducing the number of acquisitions points on the rocking curve to acquire our MIR-CT images. Although we currently acquire images at 12 points on the rocking curve, it is believed that this number may be reduced and still provide accurate images.

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PROJECT TITLE: MR Image Analysis in Multiple Sclerosis Identification of a Surrogate

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT
    Multiple sclerosis (MS) is the most common demyelinating disease in humans and has a complex clinical course that includes unpredictable relapses and variable remissions. This makes clinical evaluation of MS difficult. Therefore, current clinical trial designs must incorporate large numbers of patients followed over long periods. These designs are expensive and may deprive patients timely access to effective treatment. The use of robust surrogate marker(s) that have predictive value could reduce problems in evaluating new drugs and improve the management of individual patients. MRI-based measures such as volumes of lesions, black holes, contrast enhancements, atrophy, and magnetization transfer ratios, are expected to serve as robust surrogates. However, a number of studies have shown that the correlation between these MR measures and clinical score is weak. We hypothesize that this weak correlation is in part due to the use of improper image analysis tools necessary for robust image quantitation and in part due to a failure to define the correct MRI surrogate. In these studies we propose to develop an integrated image analysis package that is robust and automatic for accurate quantitation of tissue volumes. An important feature of this analysis package is its ability to analyze images acquired on a wide range of MR scanners using a plethora of MR sequences, greatly extending its utility. This package allows us to follow temporal changes in individual lesions, as well currently used global changes. This analysis package will be rigorously evaluated using an extensive database that contains images on more than 1,500 MS patients, followed over several years. Using this database, we propose to identify surrogate(s) based on individual or some combination of MRI-measures. Finally, this software will be distributed to a few select centers for multicenter evaluation. While the main emphasis is on MS, this system should be readily adaptable to investigate and manage various neurological disorders that require accurate determination of tissue volumes and their temporal change.

STATUS OF RESEARCH AND PARTNERSHIP
    As described in the previous report, we have developed automatic techniques for segmenting lesions, gray-matter, white-matter, and CSF. This is being validated using multi-center MRI data. The segmentation technique is based on conventional MRI sequences that are routinely used in clinical practice in MS and is machine independent. Last year we have concentrated on the automatic segmentation of black holes and high dimensional segmentation. These are described below.
    A Segmentation of Black Holes (Hypointense T1-lesions): Black holes in MS can reflect either acute edematous lesions, or matrix destruction with axonal loss on post-mortem examination. The edematous black holes generally exhibit enhancement and often resolve with time. A relatively strong correlation between change in the non enhancing black hole volume and change in EDSS was reported. There is a general consensus that black holes represent matrix destruction and axonal loss and are more harmful than the hyperintense lesions on the T2-weighted image (T2 lesions for brevity) alone and may be better correlated with clinical disability. In spite of the potential role of black holes as a surrogate in MS, hitherto identification and quantification of black holes have been largely manual processes. These

procedures introduce considerable operator bias and are not practical in handling large amount of data that is typically encountered in multi-center clinical trials. In order to overcome these limitations, we have developed, implemented, and validated a method for identification and quantification of black holes with minimal human intervention. In this method black holes are segmented on T1 images based on grayscale morphological operations. The segmented images are masked with images obtained from the orthogonalization of T2-weighted images with respect to T1 images for minimizing false classifications. The performance of this algorithm is quantitatively evaluated initially on 14 MS patients. Validation of this technique using multi-center data is in progress.

High-Dimensional Feature Map: The quality of segmentation generally improves with dimensionality of the feature space if multi-spectral images are available. However, in practice feature map analysis is most commonly limited to two-dimensions and rarely to three dimensions. Full exploitation of MR images with multiple contrasts for accurate classification of 3) feature space heterogeneous pathology may require higher dimensional (analysis. In practice, generation of feature maps in higher dimensional feature space poses severe problems because of the increased memory requirements and the associated computational complexity. For example, in the case of four dimensional feature space with dynamic intensity range of [0, 255], the total size of the feature space is 4 GB. Such large data size is extremely difficult to handle on PC's and conventional workstations. We developed and applied an efficient and novel method based on the "divide and conquer" approach for generating the high dimensional feature maps. In this method, the whole feature space was partitioned into subdivisions to deal with the memory problem. In addition, a pre-classification procedure was adopted for reducing the number of points in the feature space that need to be classified with the fast KNN (K-nearest neighbors) algorithm. As a part of the high dimensional feature space analysis, we developed and implemented a new fast KNN algorithm. This technique is implemented on a PC and applied for generating three- and four-dimensional feature maps for segmenting MR brain images of multiple sclerosis patients. Quantitative evaluation of this technique on a large number of MS patients clearly demonstrates its superiority over the two-dimensional feature space based segmentation.

Software: Together with our partner, a software package is written for image segmentation that runs on Windows platform. It is currently undergoing evaluation in our laboratory on images acquired on different scanners. The software is designed to be modular and user-friendly.

ISSUES
None.

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PROJECT TITLE: Nanotechnology Linking Biomarkers With Cancer Behavior

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute

ABSTRACT
This BRP application establishes a highly collaborative and multidisciplinary cancer nanotechnology program by integrating the bioengineering strengths of Georgia Tech (Atlanta, GA), the spectral imaging expertise of CRI (Woburn, MA), and the cancer biology and clinical oncology experiences of Emory University School of Medicine (Urology, Pathology, Radiation Oncology, Winship Cancer Institute, and the VA Hospital, Atlanta, GA). With faculty participation from eight science, engineering, and clinical departments, and advised by a prominent Scientific Advisory Board (SAB), this Partnership incorporates broad expertise in bioengineering, bioinformatics, tumor biology, bioanalytical chemistry, systems biology, hematology / oncology, pathology, and urology. Its broad and long-term goal is to develop biomedical nanotechnology, biomolecular engineering, and bioinformatics tools for linking molecular signatures (biomarkers) of cancer and the host microenvironment with cancer behavior and clinical outcome. The proposed research is broadly applicable to many types of malignant tumors such as breast cancer, colorectal carcinoma, and lymphoma, but a particular focus will be placed on the biological behavior of human prostate cancer and its clinically lethal phenotypes. A compelling reason for this focus is that prostate cancer presents a number of unique challenges and opportunities in human oncology. Its widespread occurrence (about 220,000 new cases this year in the US), tendency for a long natural history, highly heterogeneous and multi-focal histopathology, and progression to hormone independence are still poorly understood. Faced with this reality, we propose to develop advanced nanoparticle technologies (e.g., molecular beacons, semiconductor quantum dots, and enhanced Raman probes) for ultrasensitive and multiplexed profiling of biomarkers on intact cancer cells or tissue specimens. In contrast to current molecular-profiling technologies, the use of encoded nanoparticle probes allows a seamless integration of traditional pathology and cancer biology with sensitive molecular analysis, a central theme that runs across the entire proposed research. Underlying this BRP is a strong track record of the senior investigators who have worked together successfully in attracting joint research grants. In addition, the Department of Biomedical Engineering, which was jointly established in 1997 by Georgia Tech and Emory University, has presented an unusual opportunity for research collaboration to bring bioengineering technologies and discoveries into medicine and vice versa. If funded, this cancer nanotechnology program will be housed in the Winship Cancer Institute, a new 280,000 sq ft cancer research and care building located on the Emory Campus and with a truly outstanding environment for collaborative and translational cancer research. In additional to basic knowledge on cancer biology and biomarkers, this Partnership is expected to yield at least three practical outcomes: (a) a database linking molecular signatures with cancer biology and clinical outcome, (b) bioconjugated nanoparticles for molecular profiling of cancer, and (c) multiplexed spectral imaging microscopes and software.

STATUS OF RESEARCH AND PARTNERSHIP
Significant progress has been made in six key areas including (1) molecular profiling of cancer cells and tissue specimens; (2) clinical outcome database; (3) cancer biolog; (4) molecular beacon

development; (5) advanced spectral imaging; and (6) bioinformatics and data analysis. In particular, we have demonstrated that multicolored quantum dots (QDs) conjugated directly to primary antibodies (QD-Ab) can be used for simultaneous and quantitative profiling of 3 critical breast cancer biomarkers – ErbB2 (HER2/neu), estrogen receptor (ER), and progesterone receptor (PR) – in both cancer cell lines and human tissue clinical specimens. We have also correlated and validated our QD molecular profiling data with immunohistochemical staining (IHC), immuno blotting (IB), and fluorescence in-situ hybridization (FISH). Coupled with hyper-spectral imaging and wavelength-resolved spectroscopy, we have further demonstrated that multiplexed QD probes can be used to quantify a panel of up to 8 cancer biomarkers at the single-cell level, allowing correlation of traditional histopathology and molecular signatures for intact cancer cells and tissue specimens. These results raise new possibilities for nanotechnology applications in molecular pathology and clinical oncology, particularly in linking multiplexed cancer biomarkers with clinical outcome, and allowing the possibility of individualized therapy.

The work of this partnership has laready lead to a number of a joint publications in cancer anotechnology and biomolecular engineering. In particular, a high-profile manuscript has been submitted to Nature Medicine and is currently under in-depth review.

ISSUES

Issues were primarily administrative; it took longer than usual to have all the inter-institutional agreements in place.

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PROJECT TITLE: Raman Flow Cytometry for Drug Discovery and Diagnostics

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT
   The ability to make quantitative, high throughput molecular measurements of biological systems is a critical need for many areas of biomedical research. This Bioengineering Research Partnership (BRP) aims to develop a powerful new analytical platform for high throughput screening and selection based on Raman Flow Cytometry. This Partnership will develop new analytical instrumentation, optically encoded polymer resins for chemical synthesis and screening, and nanostructured materials with unique optically properties for sensitive reporting and encoding. The new technology will perform Raman spectroscopy on single particles in flow to enable new applications in sensitive multiplexed detection, drug discovery, and diagnostics. The Raman Flow Cytometry instrumentation, and applications will be developed by a Partnership involving engineers, biologists, and chemists from academia, government and industry. In the first year of the Partnership, we will modify a commercial particle sorter to detect individual Raman vibrational bands from single particles and sort these particles based on their optical signature. In Years 2-5, we will develop the ability to collect and analyze complete Raman spectra from single particles. In parallel, the Partnership will develop new encoding and reporting strategies for multiplexed molecular analysis and separation. This Raman Flow Cytometry technology will be applied to the development of therapeutics and diagnostics for bacterial pathogens and their toxins. Raman Flow Cytometry will be an important and general new analytical and separation capability that will impact many areas of basic and applied biomedical research in addition to the applications proposed here.

STATUS OF RESEARCH AND PARTNERSHIP
   The Partnership is fully functional. Sub-contracts are in place, post-docs and additional staff have been identified and hired, and excellent progress is being made on all aspects of the project.
   Instrumentation. The Partnership is developing two types of Raman Flow Cytometer. The first is an instrument that can detect discrete Raman bands from individual particles. This instrument is based on the Union Biometrics COPAS, a large particle sorter already used for the analysis of bead-based combinatorial chemical libraries. We have designed a second optical detection leg that features dispersion of scattered light via a spectrograph onto a fiber array interfaced with a bank of photomultiplier tubes. The output of the PMTs is processed with a custom designed preamp and fed back into the COPAS data acquisition system. We have characterized the performance of the instrument in terms of fluorescence sensitivity in preparation for the imminent analysis of surface enhanced Raman scattering samples. This is the primary instrumentation goal for the first two years of the Partnership. We have also begun to evaluate approaches to the development of a second type of Raman Flow Cytometer that can measure complete spectra of individual particles in flow. A separate optical bench is being used to investigate flow cell and collection optics designs, dispersive optics choices, and detectors. In addition, designs for data acquisition electronics are being developed in anticipation of increased effort on this goal beginning later in year 2 of the Partnership.

Micro- and nanoparticles. One class of applications for the Raman Flow Cytometers involves the analysis of Raman encoded microspheres. Dr Fenniri's lab at the University of Alberta is leading efforts to develop these microspheres. In the first year of the Partnership, his group completed an extensive multivariate analysis of more that 630 different polymers by hyperspectral imaging to determine suitability for the flow-based approach. They have also expanded the number of spectroscopically distinct barcodes by developing a family of halogenated polystyrene-based resins suitable not only fro Raman spectroscopy but also time-of-flight. Scale up of the synthesis of the barcoded resins with different cross-linkers in order to enhance their swelling and mechanical properties has also been acheived. Efforts to incorporate SERS-active nanoparticles to enhance the Raman spectra of the polymer have so far led to modest enhancements (~20-fold) and efforts will continue to improve this.

In addition to the analysis of the Raman spectra of polymer resins, the Partnership is developing SERS tags suitable for use as reporters (ie attached to antibodies or nucleic acid for detection) or encoding elements (incorporated into polymer particles. This effort is being led by Dr. Doorn at LANL. The LANL effort was delayed for several months due to administrative issues involving the sub-contract to Los Alamos, but these issues have been resolved and several batches of red-excited SERS tags have been characterized and appear to be suitable for these applications. These particles are based on silica-encapsulated colloidal gold aggregates bearing Raman active compounds. Efforts are underway to functionalize the silica surface to allow attachment to microspheres, antibodies, and other ligands. In addition, the Doorn group is evaluating commercial SERS particles for use in our applications.

Biological Applications. The biological applications of the Partnership involve the development and application of ligands and substrates for use in diagnostics and therapeutics for bacterial toxins and other diseases. This work is being led by Drs Nolan (LJBI) and Graves (LANL). In this first phase of the Partnership, the focus is on developing ligands and substrates for bacterial toxins using phage and other display methods, in anticipation of further optimizing these using combinatorial chemistry via the Raman-encode resins and the Raman Flow Cytometer. At LJBI, starting with cholera toxin, we have screened several phage peptide libraries for binding, and have identified a number of phage displaying peptides that bind to the toxin. These are being characterized with regard to affinity and specificity, and structural modeling will be used to develop a combinatorial chemistry strategy to further enhance these peptides. At LANL, a similar effort is underway targeting Lethal Factor, a protease from Bacillus anthracis. Once these approaches are established with these relatively well characterized targets, effort will turn to other bacterial toxins and additional targets.

ISSUES

There are no major issues confronting the Partnership at this time.

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PROJECT TITLE: Development of Networked Implantable Neuroprostheses

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke and National Institute of Biomedical Imaging and Bioengineering

ABSTRACT

Neuroprosthetic devices are powerful tools providing functional enhancement for individuals with central nervous system disorders, such as spinal cord injury and stroke. Life sustaining and improving functions such as breathing, standing, walking, grasping, reaching, micturition, and defecation have all been clinically demonstrated using neuroprostheses. Existing implanted neuroprosthetic systems utilize considerable external powering and signal processing, and each system must be customized to the specific application for which it is intended, severely limiting progress in the field and delaying the introduction of new technology to the end user. Our Biomedical Research Partnership (BRP) project addresses this issue through the development of a Networked Neuroprosthetic System (NNPS). The NNPS is based on a network of small implanted modules, distributed throughout the body, and linked to a centralized power source. The modules are networked through a network cable that distributes power to each module from a central rechargeable lithium-ion battery. Each module is dedicated to a specific function, contains processing capabilities, communicates with other modules via the network cable, and is reprogrammable over the network via a central transcutaneous link. The NNPS is extremely flexible and meets the technical needs of a broad range of neuroprosthetic applications through the selection of the appropriate modules.

STATUS OF RESEARCH AND PARTNERSHIP

We have made significant progress in the first three years of the current BRP: including: addressing critical concepts and designs that required new knowledge and/or techniques (including forming the necessary partnerships), establishing overall design topology and requirements, identifying needed system components (hardware and software), developing network communication protocols, addressing powering issues, and initiating hardware designs and prototypes.

To date, our efforts have focused on those critical aspects of the NNPS design concept that required new knowledge and/or techniques. We have: evaluated and modeled several network topologies based

upon safety, reliability, performance, ease of implementation, and the ability to upgrade; selected a network topology and identified a network communication protocol; simulated a physical layer implementation for the internal network that will simplify the segment bus interconnect mechanism; modeled component assemblies and have mocked-up critical components for evaluation; generated system level specifications; and selected technologies that are cutting edge yet are attainable within the scope of the project timeframe.

We have assembled the necessary industrial, academic and clinical partnerships in order to complete the design and fabrication of the NNPS. Thus, major areas of design uncertainty have been removed, and we believe, based on our past experience in the development of implantable systems, that we can successfully accomplish the remaining design and fabrication work.

Various configurations of networked structures have been evaluated, and the primary network topology selected. The selected network topology consists of an internal network based on a central power module with four power-transfer/data-communication multi-drop backbone leads, providing a scaleable network infrastructure for connecting multiple actuator-type and sensor-type modules. The infrastructure will support totally implanted closed-loop systems, an important goal of the distributed system concept.

We have identified the following four FES operating environments that must be addressed and accommodated by the design implementation. They include the surgical, clinical, user and research environments. The surgical installation environment limits access and communication methods. The clinical programming environment must provide transparent real-time access to all user implanted components. The user environment must have robust non-tethered operation. The research environment requires high-bandwidth communication and processing. The challenges of each environment are providing opportunities for defining and optimizing their interaction.

Although still an ongoing effort, testing for the feasibility of Li-ion rechargeable cells as the central power source for the NNPS is substantially completed. Data from our long term testing has demonstrated the suitability of the Li-ion cell for our application and, in particular, has helped us determine suitable limits and methods for charging and discharging to maximize the cell performance and lifetime.

Our project was initially funded through NINDS within which we conducted essentially a proof-of-concept study. We have recently been successful in securing an additional five years of funding through NIBIB to move the project through final system design, hardware fabrication, animal studies, and into human feasibility studies. In the later years of the project, we intend to realize a first configuration of the NNPS in individuals with spinal cord injury to provide enhanced grasp/release. This human feasibility study will provide the foundation for broader clinical application of the NNPS.

We believe that the NNPS is a revolutionary contribution to the field of neuroprosthetics; it is easily configured for current and anticipated neuroprosthetic applications, accommodates new innovations by participants in the field, and eliminates external components, and can be easily implemented using current surgical techniques.

ISSUES

Technical: In general, our major task is one of large system integration; identifying, evaluating, modifying, and combining the wide variety of technical aspects needed for the networked system. However, there is significant work to be done in creating and developing one or two of the technical components. In particular, developing and fabricating a new class of leadwire and interconnect that can be used to form the network infrastructure will be one of the most challenging.

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PROJECT TITLE: 3D Imaging of Electrical Activity in Myocardial Tissue

PARTNERS' NAMES AND AFFILIATIONS:

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT

Understanding the mechanisms that underlie abnormalities of electrical conduction in the heart is the key to the development of effective antiarrhythmic therapies. During the last decade, significant progress has been made in imaging electrical excitation waves in the heart using voltage-sensitive fluorescent dyes. However, until recently such imaging was limited primarily to the epicardial surface. Our goal is to develop a technology that would enable optical imaging of electrical excitation throughout the myocardial wall and 3D visualization of the organizing centers of vortex-like electrical activity (filaments) involved in the initiation and maintenance of ventricular fibrillation. To address the technical challenges of this new technology we coordinated efforts of the research groups of Dr. A. Pertsov, who pioneered the 3D imaging of vortex-like excitation in chemical excitable systems and in the heart; Dr. D. Boas, an expert in optical tomography; Dr. L. Loew, a leader in the development of voltage-sensitive probes and optical imaging; and Dr. D. Weitz, renowned for his expertise in multiple-scattering media. The specific aims of the project are: 1) to create realistic computer models for reconstructing 2D optical images from 3D distributions of the transmembrane potential in myocardial tissue (forward problem), 2) to apply diffusive optical tomography to 3D reconstruction of the actual electrical activation in the heart (inverse problem); 3) to design, synthesize and test in myocardial tissues a family of near-infrared voltage-sensitive dyes optimized for 3D imaging of electrical activation in the heart; 4) to explore two-photon fluorescence and second-harmonic generation for 3D imaging of electrical activity in cardiac myocytes and tissues at sub-cellular and sub-millimeter scales. Successful completion of this project will break ground for a new technology, the 3D imaging of electrical activation in the heart.

STATUS OF RESEARCH AND PARTNERSHIP

Specific Aim 1. We have completed measurements of light absorption and scattering in myocardial tissues stained with the voltage-sensitive dye di-4- ANEPPS. Based on these measurements we have developed a two-stage model in which the output of a 3D ionic model of electrical excitation serves as input to an optical model of light scattering and absorption inside heart tissue. Using this model we assessed different optical tomographic methods with respect to their effectiveness in visualizing 3D cardiac activity. Specifically, we considered scanning and broad-field illumination, including trans- and epi-illumination. Optical diffusion theory was applied to derive a computationally efficient approximation of the point-spread function and to predict voltage-sensitive signals. Computations were performed both for fluorescent and absorptive voltage-sensitive dyes. Among all the above-mentioned methods, fluorescent coaxial scanning at appropriate wavelengths yields the best lateral optical resolution (<2.5 mm) and signal intensity, both as functions of the source depth. These findings set the stage for the next phase of the proposed studies by identifying the most promising experimental approaches to

visualizing 3D cardiac activity. During the next year we shall extend our studies from simple slab geometry to realistic geometries of the myocardial wall.

Specific Aim 2. We worked on deconvolution approaches for imaging of intramural sources of excitation including scroll wave filaments - the organizing centers of 3D reentrant activity, responsible, as stated above, for the most dangerous cardiac arrhythmias. We have developed a novel method for the localization of cardiac electrical waves based on integrated signal ratios. Using the proposed algorithm, we have shown that the depth of point sources can be accurately determined even in the presence of significant noise. In the case of expanding ellipsoidal waves, the method allows good depth estimates for their outer boundaries.

We have begun to work on the development of an experimental laser scanning system for 3D imaging of electrical propagation. The preliminary experiments using phantom models, as well as in live tissue preparations, have been conducted at Harvard and at SUNY, respectively. During the next year we will compete the development of a prototype laser-scanning imaging system and will start testing the system and the reconstruction software using phantom models and live tissue preparations.

Specific Aim 3. We continued working on design, synthesis and testing in myocardial tissues of a family of near-infrared voltage-sensitive dyes optimized for 3D imaging of electrical activation in the heart. Our original strategy for delivering dyes of the styryl class was to encapsulate near-infrared styryls in gamma-cyclodextrin to improve their aqueous solubility and thereby permit deeper penetration. We tested an array of cyclodextrin complexes of these dyes and also included di-8-ANEPPS, a more hydrophobic version of di-4-ANEPPS, as a test case with a known high voltage sensitivity, but very poor solubility. The cyclodextrin complexes did indeed prove to be water-soluble. However, when the complexes were tested in live tissues, staining never penetrated past the endothelial layer.

As an alternative strategy we have developed probes with double positive charges so that their intrinsic solubility was higher. We have prepared and tested 3 dyes with hydrocarbon side chains of varying lengths: JPW-5020, JPW-5034, JPW-3067. All 3 of these dyes gave strong voltage-dependent signals in the near infrared when tested on hemispherical bilayer apparatus and in the heart. The largest sensitivity was displayed by JPW-5034 with a fluorescence change of 4%/100mV for emission taken with a 850nm long-pass filter. The other class of dyes we have been working on contain oxonol chromophores. These are expected to display absorbance maxima in the 700-800nm range. During the next year we will develop and start testing long wavelength oxonols with a heptamethine linker between the heterocyclic nuclei. We will adopt a strategy of using a cyclic bridge to stabilize the intermediate.

Specific Aim 4. We obtained SHG images of isolated rat cardiac myocytes stained with several dyes. We have established the line scan mode for recording the voltage-sensitivity of the SHG signal. Thus far, we have had difficulty extracting the signal from motion artifacts due to the contraction accompanying action potentials. We have just recently been able to co-label cells with both the SHG dyes and a calcium indicator, Fluo-4, and have obtained good calcium action potentials and SHG signals. We are currently modifying the optics to allow acquisition of both signals simultaneously. During the next year we will complete line scan studies of SHG on cardiomyocytes using simultaneous recording of calcium signals. We hope to be able to apply patch clamp control and recording of the action potentials to best establish the voltage sensitivity of the SHG signal. We are also working on a fast wide-field SHG microscope that we will test with the cardiomyocyte preparation. If successful, these experiments will significantly improve the accuracy of the measurements of spatial and temporal distribution of the transmembrane potential at subcellular resolution in cardiac myocytes and other types of excitable cells.

ISSUES
None.

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PROJECT TITLE: Multi-keV X-Ray Microscopy Facility for Bio-imaging

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT
We propose to develop a zone plate based, multi-keV x-ray imaging facility at the Stanford Synchrotron Radiation Laboratory (SSRL) that will be capable of imaging thick hydrated biological specimens with 20 nm resolution and provide three dimensional images using tomographic reconstruction. By using x-ray phase contrast imaging as well as contrasting agents and specific labels such as immunogold, quantum dots, or other labeling techniques developed for electron microscopy, the instrument will be a tool for mapping specific molecules in cells or cells in tissues. With the anticipated improvements in zone plate optics, we expect the resolution to ultimately reach or exceed 10 nm. This instrument is meant to be used by the biomedical research community at large, and therefore in its construction we will demonstrate its efficacy on a biological problem to determine the nanostructure of hydrated bone. This proposal combines the expertise of three collaborating organizations: Prof. P. Pianetta and Dr. K. Luening from SSRL with extensive experience in a wide range of x-ray techniques based on synchrotron radiation sources and Dr. E. Almeida (UCSF, NASA Ames Research Center) and Prof. M. van der Meulen (Cornell University) who are leaders in studies of the nanostructure of bone. The microscope will be purchased from Xradia Inc., a leader in developing nondestructive high-resolution x-ray imaging solutions using multi-keV x-rays produced by a laboratory x-ray source. Dr. Wenbing Yun, founder and president of Xradia, will serve as a consultant on the project to develop the techniques needed for achieving high resolution 2D and 3D images in phase contrast for real biological specimens.

STATUS OF RESEARCH AND PARTNERSHIP
The project is in its first quarter of FY01 so the primary goal is to define the detailed optical and system configuration of the x-ray microscope so an order can be placed for the instrument by the beginning of the second quarter. At present two optical configurations are being studied, the originally proposed configuration in which the condenser optics refocuses the beam onto the sample from a real focus located between 0.5 to 1 m upstream of the microscope and a new configuration in which the condenser operates on a virtual focus approximately 0.5 m downstream of the sample. This configuration is being considered because it reduces the overall length of the instrument and may provide some advantages with respect to beam stability. Ray tracing studies are being performed on these two configurations to determine if consideration of the second option is warranted. The specifications for the microscope are being written and will incorporate the new ray tracing information as it is developed. The x-ray microscope is being designed to include easily interchangeable, multiple condenser optics and zone plates in order to cover the proposed energy ranges as well as the phase contrast capabilities. The beam line which will provide the photons to the x-ray microscope has been rebuilt with state of the art optics and commissioned as has the entrance aperture system.

Although the funding for the partner institutions is not scheduled to begin until FY02, the first meeting of the entire team is being planned for the second quarter of FY01 to review the progress to date and the future plans as well as to make sure that samples are ready for imaging as soon as the instrument is installed.

ISSUES
None.

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PROJECT TITLE: Single Cell Analysis Using Microfluidic Platforms

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GRANTING NIH INSTITUTE/CENTER: National Human Genome Research Institute

ABSTRACT
Purpose. This partnership will bridge the technology development efforts of Caltech faculty in Applied Physics and Bioengineering with clinical needs of faculty at the USC Medical School. We will apply technology for nanofluidic chips that has been developed at Caltech to problems of biological and medical interest. We will develop microfabricated chips with the ability to manipulate nanoliters of fluid; these chips will be used to perform highly parallel biochemical manipulations and genetic analyses of rare populations of cells. We will use the chip technology to attack the following two problems of particular medical importance: factor and marker discovery in hematopoeitic stem cells, and the discovery and characterization of unculturable pathogens in the human gut.

Methods. We will utilize multilayer soft lithography to fabricate our devices. We will then develop on-chip assays with standard reagents that work successfully by conventional off-chip methods. We will make modifications to the assays as we see fit, in order to create the most optimal chip-based system. In order to implement integrated heating and cooling, we will fabricate micro-peltier junctions.

Results. We developed a chip capable of generating cDNA from single cells and small numbers of cells in a parallel fashion. We used this chip to trap, lyse, purify mRNA, and synthesize 1st strand cDNA from various samples of 3T3 cells and sub pg quantities of mRNA. We calibrated our chips by utilizing the processed samples as templates for quantitative PCR (qPCR) and then comparing threshold cycles to unprocessed 3T3 mRNA templates. We also utilized the chips to extrapolate gapdh copy number from single and small numbers of cells. The qPCR calibrations for mRNA isolation and cDNA synthesis proved our method to be reliable and reproducible for 3T3 mRNA templates as small as 0.1 pg. Our efficiency for the processes of mRNA isolation and mRNA isolation/cDNA synthesis was 80% and 44%, respectively.

We also used microfluidic PCR chips to analyze the microbial ecology of the termite gut. Our results from gut contents demonstrate the ability to discover the prevalence and ribotypes of FTFHS expressing bacteria that cannot be analyzed by conventional culture methods. Furthermore, preliminary results indicate our detection is digital, which will allow for future analysis of mRNA copy number in single cells.

Our coworkers at USC developed a high-throughput multi-antigen microfluidic fluorescence immunoassay system. A 100-chamber PDMS (polydimethylsiloxane) chip performs up to 5 tests for each of 10 samples. In the particular study system, specificity of detection was demonstrated and calibration curves were produced for C-Reactive Protein (CRP), Prostate Specific Antigen (PSA), ferritin, and Vascular Endothelial Growth Factor (VEGF). The measurements show sensitivity at and below clinically normal levels (with S/N > 8 at as low as 10 pM antigen concentration). The chip uses 100 nL per sample for all tests and represents a technological advance for scientific research and "point-of-care" testing in medicine.

Finally, we have also developed refrigeration and heating systems for rapid thermal management of nanoliter fluid volumes with micro-Peltier junctions. The temperature of small liquid reservoirs can be rapidly changed and controlled within a range between -3 °C to over 100 °C with good long-term stability. These thermal management systems enable the fabrication of complex chip-based chemical and bio-chemical reaction systems in which the temperature of many processes can be controlled independently.

Conclusions. The results the collaborators obtained show that we are on track to achieve our major goals and that the partnership is a fruitful one.

STATUS OF RESEARCH AND PARTNERSHIP

The results presented in the abstract demonstrate the ability of the partners located at Caltech, Stanford and USC to work together in a productive, successful manner.

ISSUES

The main issues and challenges will come when we integrate processes (PCR, lysis, detection) on more complicated chips. Each step will need to be optimized separately and then together.

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PROJECT TITLE: Micro-Electric Impedance Spectroscopy of Inner-Ear Hair Cells

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Institute on Deafness and Other Communication Disorders

ABSTRACT
    This project is aimed at the development and testing of micro-electric impedance spectroscopy (microEIS) and tomography (microEIT) hardware and reconstruction software to record and image the spatio-temporal distribution of electrical properties within the cytoplasm, organelles and membranes of vestibular and auditory sensory hair cells. A combination of flex-circuit technology and standard lithographic microfabrication techniques are used to construct micro-recording chambers instrumented with arrays of metal electrodes at subcellular dimensions. Isolated cells are positioned within the instrumented recording zone under mircroscopic observation and interrogated using radio frequency electrical signals. Voltage and current are measured around the outside surface of the cell and used to reconstruct three-dimensional maps or images of the conductivity and permittivity throughout the cell. MicroEIT systems are being used to interrogate electrical properties of cochlear outer hair cells and type II vestibular hair cells in response to micromechancical cilia displacements, electrical stimuli, and chemical stimuli. Results are contributing to our fundamental understanding of the spatial distribution and temporal response of electrical properties in these important sensory neurons. Perhaps more importantly, microEIT devices developed as part of the research, are providing an entirely new window through which to view the living machinery of a wide variety of normal and pathological cells. The project integrates bioelectricity, imaging, bioinstrumentation, micro/nano-bioesensors, physiological modeling/computation, biomechanics and microfluidics. Devices involve on-chip transport of solutions/pharmaceutics and living cells

STATUS OF RESEARCH AND PARTNERSHIP
    The project is currently in the fourth year of funding (R01 DC04928, start date: August 2001). All subcontracts were established within the first month of the grant. The scientific and engineering aims of the project are proceeding as outlined in the proposal. We have fabricated over a dozen unique wafer designs, each including approximately 40 useful microdevices of various sizes and layouts. Due to the small scale of the devices and high interrogation frequencies employed, considerable attention has been devoted to the development of reliable, user friendly, microfluid and electrical interconnects. We have developed two types of quick-connect fluid-mechanical interfaces that greatly simplify practical use of the micro-EI chips. The interfaces include on-board RF computer-controlled head-stage FET amplifiers and reference impedances. Each interface is directly connected to a bank of computer controlled arbitrary waveform generators and digital scopes that allow a great deal of flexibility in experimental design, data acquisition and analysis.
    We have used microEI developed under BRP funding to investigate electromotility of cochlear outer hair cells (OHCs) at unprecedented temporal speed and spatial resolution. Results demonstrate, for the

first time, wave propagation along the lateral wall of the outer hair cells and high-frequency electrical resonances. The fundamental resonance frequency averaged fn~13kHz (Q~1.7). Higher-order resonances were also detected. Resonances were ultrasonic relative to the characteristic best frequencies in the region of the cochlea from which the cells were isolated. Results have implications regarding OHC function and regarding the role of the motor protein prestin in the exquisite selectivity and sensitivity of the mammalian cochlea. We have also used microEI to study the spatial distribution of passive dielectric properties in a wide variety of cell types. Preliminary data indicate the presence of previously undetected dielectric dispersion in cell membranes that is particularly pronounced at radio frequencies. Cardiac myocytes have also been used in preliminary studies. Rapid changes in membrane conductances during active contraction were readily apparent using radio-frequency microEI. These data illustrate the potential of the technology for micro electric-impedance tomography at subcellular dimensions. Inventions derived from this work have been disclosed to the Technology Transfer Office at the University of Utah. Some of the technology has been patented and a license is under negotiation for use in automated hematology analysis. The company E.I. Spectra (H.E. Ayliffe; Seattle WA) was founded on the basis of the BRP effort. E.I. Spectra has been successful in securing initial start-up funding. In summary, we have succeeded in developing, applying, and translating microEI technology. We are currently applying this new technology to address questions of importance to health and the human condition, with specific focus on sensory hair cells of the inner ear.

ISSUES

We have not experienced any serious issues. With regard to the future of BRPs, there are concerns regarding: 1) how to fund projects that will require more that 10 years of effort, 2) relative importance of patents and tech-transfer vs. scientific publications in the review of BRP renewals, and 3) the relative efficiency of large BRPs vs. smaller independent investigator led projects.

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PROJECT TITLE: Nanotechnology for the Structural Interrogation of DNA

PARTNERS' NAMES AND AFFILIATIONS:
Arthur P. Baddorf, Robert S. Foote (Oak Ridge National Laboratory); Shengting Cui, Hanno Weitering (University of Tennessee); Massimiliano Di Ventra (University of California, San Diego); Leonard C. Feldman (Vanderbilt University); J. Thomas Dickinson (Washington State University)

GRANTING NIH INSTITUTE/CENTER: National Human Genome Research Institute

ABSTRACT
We propose a research program to achieve the goal of sequencing of single molecules of polynucleotides using conductance probes within a molecular scale aperture and to demonstrate the technical feasibility of this promising approach. There have recently been intriguing suggestions about how one might rapidly determine the sequence of a single DNA molecule contained in a buffer solution by transporting it through a voltage-biased nanoscale aperture while monitoring the ionic current through that aperture, e.g., Kascianowicz and Deamer. Some suggestive proof-of-principle experiments have been demonstrated using lipid bilayer supported protein pores and observing variations in pore axial conductance. We contend that for this strategy to become a realizable technology, robust nanometer scale apertures must be fabricated using a combination of top-down and bottom-up approaches. In addition, interesting variants of this approach such as incorporating laterally opposed nanoelectrodes in a nanochannel for probing monomeric variations in the electrical properties of polynucleotides can only be achieved through nanofabrication. Our specific aims include the following, 1) develop fabrication capabilities that combine top-down and bottom-up strategies for forming fluidic channels and electrical probes with length scales approaching 1 nm, 2) investigate the dependence of the length scale probed on nanopore axial and lateral dimensions, 3) determine impact of polymer dynamics on fundamental limits of DNA structural determinations.

STATUS OF RESEARCH AND PARTNERSHIP
Our efforts over the last nine months, since the project began, have advanced on all fronts including theoretical understanding of ion and DNA transport through nanopores, transverse electronic transport through ssDNA, in addition to nanopore, nanochannel, and nanoelectrode fabrication. One of the more crucial questions regarding our strategy of electrically probing the individual bases of an intact piece of ssDNA is whether the tunneling current signatures of the four individual bases are distinguishable. Di Ventra's group has theoretically investigated charge transport in ssDNA in the direction perpendicular to the backbone axis. He has found that, if the electrodes which sandwich the DNA have the appropriate spatial width, each nucleotide carries a unique signature due to the different electronic and chemical structure of the four bases. This signature is independent of the nearest-neighbor nucleotides. Furthermore, except for the nucleotides with Guanine and Cytosine bases, the difference in conductance of the nucleotides is found to be large for most orientations of the bases with respect to the electrodes. This is the first study ever of charge transport in DNA in the transverse direction and lends support to the idea that sequencing DNA using nanopores with lateral electrodes can indeed be an effective tool for the electronic read-out of single bases.

Moreover, theoretical work has been carried out by Cui to establish a foundation for the understanding of ion mobility in cylindrical pores, which is obtained through the diffusion coefficient. This work shows that the classical Fick's law diffusion equation can be used to obtain the average diffusion coefficient of molecules in cylindrical nanopores in the size range 1.5 to 3.0 nm for both axial and radial directions. The work thus establishes the theoretical framework for the calculation of the transverse diffusion coefficient and ion mobility, and hence the ionic current in the transverse direction of nanopores. A paper describing some of this work has been accepted for publication in the Journal of Chemical Physics. We carried out molecular dynamics calculations to investigate the ion diffusion in nanopore of about 2 nm in the presence of single stranded DNA. The preliminary results suggest that the ion diffusion is faster in the presence of [A20] than [C20].

The UNC group has been developing techniques for forming nanopores in aluminum oxide, silicon oxide and silicon nitride nanoscale membranes. We have developed process strategies for reliably fabricating silicon oxide membranes with thicknesses of 20-40 nm supported by silicon substrates. We have also fabricated similar silicon nitride membranes. Attempts at focused ion beam (FIB) milling nanopores (by the Feldman group at Vanderbilt) into such windows resulted in minimum diameters in the 30-40 nm range which are not useful for single molecule detection directly. Prior efforts by Mochel led us to investigate the use of energetic focused electron beams to form small features in nanoscale membranes. We have been successful in forming pores in alumina and silicon nitride with diameters less than 2 nm using electron beam milling. More interestingly, we have developed a proprietary method for reshaping silicon nitride using focused electron beams. This material manipulation technique has been used to form pores reduced in size to <2 nm in addition to forming electrode-like structures.

Baddorf and Jesse at ORNL have been developing a fabrication strategy for forming electrodes consisting of carbon nanotubes (CNT) or nanowires inside of nanochannels. Opposing microscale metal electrodes have been photolithographically patterned on an oxidized silicon wafer. An ac electric field is used to dielectrophoretically manipulate CNTs that are contained in a solution deposited onto the electrodes. The nanotubes orient normal to the edge of one metal electrode and eventually bridge the gap between the opposing electrodes. Electrical contact between the metal electrodes by the CNTs is detected by monitoring electrical conductance. CNT bridging has been achieved by bundles of CNTs with diameters of 10-20 nm. Solution characteristics are being modified to eliminate agglomeration of the CNT bundles so that bridging can be accomplished by a single CNT.

The group of Feldman at Vanderbilt University has been working with UNC, as noted above, and with the ORNL group for fabrication of nanochannels containing CNT electrodes. They have also continued their efforts to utilize multilayered heterostructure materials for the formation of nanochannels. This strategy involves using FIB milling to expose the oxide layer of a silicon-on-insulator (SOI) wafer. The oxide layer is then chemically etched to create a channel and the channel closed using oxide deposition or silicon collapse. Channels have been formed in SOI wafers with a 50-nm oxide layer, thus a 50-nm wide channel with a depth controlled by the etching process. The Dickinson group at Washington State University has been exploring the ability to obtain submicron lateral resolution with their AFM induced chemical mechanical polishing technique. Materials being explored include soda lime glass, brushite, gypsum, sapphire, and mica.

ISSUES

There have been delays of several months in establishing a subcontract with ORNL that has limited their progress.

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PROJECT TITLE: Single Cell Multiplex Screening of Protein Families

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT
   This proposal seeks to develop a Novel Single Cell Target Family high throughput screening (HTS) platform that will be uniquely capable of screening entire protein target families simultaneously. The following advantages will be realized due to its maximized efficiencies: i) improved drug selectivity and a reduction in drug side effects, and ii) greater than ten fold reduction in the cost of screening and drug candidate optimization. Additionally, novel instrumentation and methodologies will become available to the biomedical research community for academic and industrial use, including a high speed, six laser flow cytometer developed through DakoCytomation the (BRP partner) and an automated tissue culture system developed through The Automation Partnership. The Specific Aims are i) to assist in development of and procure an automated tissue culture system capable of handling over 300 microwell plates and nearly 2,000 distinct cell lines, ii) to expand the capabilities of our automated sampling system for flow cytometry (FCM) for greater speed and to enable unique analysis and sorting modes, iii) to develop at least 150 agonist-responsive GPCR target cell lines by pioneering a novel Target Family Assay Development approach based on Cellular and Molecular Evolution, iv) to develop a single cell HTS screening platform based on six-laser FCM, multiparametric detection technology and an automated cell preparation system, v) to perform a focused library test screen of 10,000 compounds against the 150 GPCR targets, and vi) to develop the database and bioinformatics tools to integrate and help interpret the data. When fully integrated, tested and validated the system will be applicable to many target families of relevance to biomedical and pharmaceutical research, such as other receptor classes, and will have a broad range of drug discovery applications.

STATUS OF RESEARCH AND PARTNERSHIP
Year 1, Status of Research and Partnership:
   Aim 1, Year 1. This Aim was to include The Automation Partnership (UK) as a second partner, but was deleted from the grant upon funding as Novasite agreed to seek other means to support this costly component of the project. An automated Tissue Culture system remains an important component of the overall project since the maintenance and preparation of 150 distinct populations suitable for real-time viable cell assays is an onerous task and an automated system represents a substantial cost savings and improvement in accuracy as compared to the cost of 10 full time technical staff performing the tasks manually. So, progress is described here. We have been exploring the options of either modifying existing systems that were designed for other applications to meet our needs, which approximately doubles the cost, or developing a dedicated system from the ground up. The latter option would involve purchasing hardware from vendors and developing software components to control the system, but it allows us to build the system functionality specifically for our needs and to integrate expansion options for anticipated future needs. We will select a development path in the next 12 months.
   Aim 2, Year 1. This Aim has been achieved. Two-Arm DSIS automated mixing and injection system is highly flexible through software and hardware modifications. One arm is used to mix cells with test compounds in microwell plates and the second is used to inject the mixture to the flow cytometer (FCM).

The mixing process uses adjustable volumes of cells and compounds, and the number of mixing steps is selectable. Compounds can be taken from plates or tubes arranged on the deck. Different compounds can also be added to the same set of cells, allowing for pre-incubation with one test compound before exposure to a second, and the exposure time in the first compound can be delayed from seconds to minutes without slowing the sampling rate. This allows us to investigate compound behaviors in agonist or antagonist mode, and also enables investigation of allosteric modulators. The injection step incorporates user selectable variation in the rate and volume of sample introduction to the FCM and in the amount of time that elapses after mixing before the sample is injected. This allows interrogation of a range of response kinetics from seconds to minutes which allows us to evaluate a range of responses that derive from a diversity of G Protein-Coupled Receptor (GPCR) targets and a diversity of compound affinities and efficacies. We have also demonstrated that the system can faithfully sort (isolate) desired cells and deposit them into either tubes or microwell plates as accurately as traditional sorting procedures. This feature has been used towards completion of Aim 3 (Assay Development) to isolate (clone) single cells from heterogeneous target-transfected starting populations in which less than 2% of the cells respond to the cognate ligand. The selected populations or clones are then grown and re-evaluated, and response frequencies are elevated to 30-90% in a single pass. Software has been developed that permits fully automated determination of the frequency of cells responding in over 150 gated populations, yielding signal to background ratios of over 50 fold and extremely high resolution for detecting weak activities.

   Aim 3, Years 1-3. The target cell currently is HEK293, and many of the tested GPCRs transfected will generate a Ca2+ response after exposure to the natural ligand in a fraction of the stable cells. A few GPCR do not couple directly, and a series of stable cell lines have been established to enhance coupling to the Ca2+ pathway (e.g. promiscuous G protein constructs.) Intractable GPCR transfected into alternative lines have yielded detectable Ca2+ responses. The native control or promiscuous G protein bearing lines have been exposed to a subset of over 30 natural ligands to determine whether any of the GPCR targets occur endogenously in HEK293, and this has been repeated with 10 other cell lines to find alternative cells lacking the target GPCR. Only one transfectable alternative cell has been required to build alternative GPCR assays. cDNA clones have been purchased from Origene (Rockville, MD), subcloned as needed and over 80 GPCR bearing cell lines have been entered into development. The Automated DSIS is used to sort and develop the optimally responsive populations or clones.

   Aim 4, Years 1-3. We have explored new options for developing multiplexed populations since the proposal was written and have found that with 2 lasers we can resolve 87 target populations and appreciate a Ca2+ response. With a 3rd laser we anticipate increasing that number by 50% or more, although we can also foresee some potential limitations. Thus, we anticipate requiring at most 4 lasers to identify the 150 populations specified in the proposal with the 5th laser being used to excite the Ca2+ sensing probe. Due to this development we have focused more effort in Year 1 on characterization and optimization of the color-coding techniques using existing laser systems and will begin design specifications for a 5 laser FCM with DakoCytomation in Year 2. As the number of addressable assay populations grows, the need for an automated system for consistent and reproducible staining of the populations is apparent. This need will be addressed immediately in Year 2 through the development of a Tecan Evo liquid handler that can accept multiple cell populations and deliver accurately stained cells for entry into the Automated DSIS.

   Aims 5, 6. The database development and screen will occur in Years 3 and 4, respectively.

ISSUES
   None. Our partner, DakoCytomation, has been very responsive to requests for contributions regarding system development.

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PROJECT TITLE: High Field MR Research in Drug Abuse: A Bioengineering Research Partnership

PARTNERS' NAMES AND AFFILIATIONS:
Brain Imaging Center, Behavioral Psychopharmacology Research Laboratory, Developmental Biopsychiatry Research Program, and Translational Imaging Laboratory, McLean Hospital (Belmont, MA); Bioengineering Center, Department of Electrical Engineering and Computer Science, Tufts University (Medford, MA); Department of Psychiatry, Boston University School of Medicine (Boston, MA); Department of Psychiatry, University of New Mexico (Albuquerque, NM); Seoul National University (Seoul, South Korea - added in 2004)

GRANTING NIH INSTITUTE/CENTER: National Institute on Drug Abuse (R01 DA14178)

ABSTRACT
Magnetic resonance spectroscopy (MRS) and functional magnetic resonance imaging (fMRI) are promising new imaging modalities that are increasing our understanding of the nature of drug abuse and addiction. This bioengineering research program involves a total of 15 engineering projects that will enhance the capabilities of this unique magnetic resonance research center to conduct studies both of individuals with substance abuse disorders as well as studies using animal models of substance abuse disorders

STATUS OF RESEARCH AND PARTNERSHIP
1. With support from the Office of National Drug Control Policy and the National Center for Research Resources, McLean Hospital purchased a Varian 9.4 Tesla 40 cm horizontal bore animal scanner. That system which will help us to extend our translational animal addiction imaging program to include rodents and small nonhuman primate species. The system is projected to become operational in the 3rd quarter of 2006, and it will offer superior spatial and spectral resolution compared to our 4T and 3T systems. The ultra high magnetic field strength will allow us to conduct imaging, fast imaging, and spectroscopy studies in the rodent brain, which is less than 1% of the volume of the human brain, and will allow us to conduct multinuclear spectroscopy studies in anatomically relevant volumes in nonhuman primate and rodent brains. The ultra high field strength of this system presents unique bioengineering challenges. We are refocussing many of our bioengineering projects on enhancements including subject restraint devices, noise-attenuating systems (to minimize scanner noise-induced stress in unanesthetized subjects), stimulus presentation devices, new coils, and physiological monitoring hardware, to support studies in awake and anesthetized subjects. In addition, we will be working to translate all technology enhancements already supported by this program for our 3T and 4T systems to the 9.4 T scanner, so that we can acquire in animal subjects most scan types we are able to acquire in humans. This will permit our program to truly be translational. We anticipate that these bioengineering challenges will be a primary focus for our technology development in future years of this program.
2. We have made substantial progress with 3T functional MRI studies of unanesthetized macaques (cynomolgus monkeys) and squirrel monkeys. These two species present different challenges because of their different brain and body sizes. With macaques (brain volumes ~80 cm3), we documented robust and reliable BOLD fMRI activations in response to visual stimuli, noxious thermal stimuli, morphine infusions, and opioid antagonist (naloxone) infusions. We plan to continue these studies to characterize several novel opioid medications to determine brain substrates associated with analgesia and abuse liability. In squirrel monkeys (brain volumes <30 cm3), we acquired good quality proton

spectra and proton magnetic resonance spectroscopic imaging data from unanesthetized squirrel monkeys. We are continuing hardware and software development efforts to improve data acquisitions from both nonhuman primate species. As noted above, these efforts are being developed so that they can be ported to the 9.4 T system once it becomes operational.

ISSUES

The Varian Unity/Inova 4T scanner had several hardware upgrades during this project year including a swap-in of a new, 800V MTS Gradient Amplifier Power Supply, which is performing better than the prior hardware and providing more stable gradient performance. In addition, a power-conditioning UPS system was just installed which should improve both power stability to the system and system performance.

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PROJECT TITLE: Optical Molecular Imaging of Cancer

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute

ABSTRACT

Cancer is a major public health problem. Currently, classification of cancer is based on phenotypic markers. The identification of unique molecular markers of cancer has led to development of new molecular cancer therapies. Movement toward a molecular characterization of cancer would have important clinical benefits, including (1) detecting cancer earlier, (2) predicting risk of precancerous lesion progression, (3) detecting margins in the operating room in real time, (4) selecting molecular therapy rationally and (5) monitoring response to therapy in real time at a molecular level. Imaging the molecular features of cancer requires molecular-specific contrast agents which can safely be used in vivo as well as cost-effective imaging systems to rapidly and non-invasively image the uptake, distribution and binding of these agents in vivo. Radiographic imaging modalities such as CT and MRI, although useful for delineating the deep extent of advanced carcinomas, are not sufficiently sensitive to detect small, intraepithelial lesions. Optical imaging is a new modality which enables real time, high resolution imaging of epithelial tissue. Optical imaging systems are inexpensive, robust and portable. Optical imaging systems are ideally suited for early detection of intraepithelial disease and to assess tumor margins and response to therapy.

The goal of this proposal is to integrate development of optical imaging systems and contrast agents with advances in functional genomics. We will develop molecular-specific, optically active contrast agents that can be applied topically. We will also develop inexpensive, rugged and portable imaging systems to monitor the three-dimensional profile of targeted biomarkers. These contrast agents and imaging systems will have broad applicability to many types of cancer; here, we will develop and test agents and imaging systems for the cervix, oral cavity and the lung, which represent more than 20% of both tumor incidence and mortality worldwide. We will test the safety and efficacy of these contrast agents and imaging systems in animal models, providing data to support phase I and II clinical trials. The aims of this proposal are to: (1) Develop optically active contrast agents to target four molecular signatures of neoplasia, including v integrin; (2) to identify promising new biomarkers EGFR, MMP, telomerase and for which contrast agents will be developed using SAGE libraries, and to identify promising molecular probes for novel contrast agents using combinatorial methods; (3) to develop inexpensive, portable optical systems to image the morphologic and molecular signatures of neoplasia noninvasively in real time; and (4) to test these agents, delivery formulations and imaging systems in living biological systems of progressively increasing complexity. (5) Our final aim is to integrate these studies to develop a miniature imaging system, which when coupled with the contrast agents developed here, can be used for real time, molecular detection of neoplasia and to monitor, at the molecular level, whether a lesion is responding to therapy.

STATUS OF RESEARCH AND PARTNERSHIP

We are carrying out tests of contrast agents in cell lines, tissue culture and in ex vivo human surgical specimens. We are beginning tests of contrast agents in animal models. We are preparing to submit an IND application to the FDA for approval for phase I clinical trials of our first agent.

ISSUES

Our biggest challenge is to maintain active communication across multiple institutions and cities. We have found NetMeetings and student exchange solve some of these issues.

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PROJECT TITLE: Integrating Data, Models, and Reasoning in Critical Care

PARTNERS' NAMES AND AFFILIATIONS:
Massachusetts Institute of Technology (Cambridge, MA), Beth Israel Deaconess Medical Center (Boston, MA), Philips Medical Division (Andover, MA)

GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT

The broad objective of this Bioengineering Partnership is to focus the resources of an interdisciplinary partnership from academia (MIT), industry (Philips Medical Systems), and clinical medicine (Beth Israel Deaconess Medical Center) to develop and evaluate advanced ICU patient monitoring systems that will substantially improve the efficiency, accuracy, and timeliness of clinical decision-making and improve patient outcome.

Modern intensive care units employ an impressive array of technologically sophisticated instrumentation to provide detailed assessment of the pathophysiological state of each patient. Ideally, such monitoring permits the early detection of changes in the patient's condition and provides information that both supports therapeutic decision-making and assists in evaluating the response to treatment. However, providing life support in the ICU is becoming an increasingly complex task because of the growing volume of relevant data from clinical observations, bedside monitors, mechanical ventilators and a wide variety of laboratory tests and imaging studies. Furthermore, the available data is typically scattered among different computers, data-bases, hand-written physician and nursing records, and waveforms/trend-plots generated by bedside monitors. The enormous amount of ICU data and its poor organization makes its integration and interpretation time-consuming and inefficient, and has created information overload, which may lead to errors and mishaps in ICU care. On the other hand, the richness and detail of the collected data make it feasible to utilize the power of modern signal processing, pattern recognition, computational modeling, and expert systems to conduct real-time tracking of the pathophysiological state of the patient, to produce hypothesis-driven graphical user interfaces, to reduce the incidence of false alarms, and to support early recognition of important physiological trends that will permit earlier therapeutic intervention.

This research effort involves the collection and annotation of an extensive and comprehensive new database from ICU patients to support research in intelligent patient monitoring. The growing database currently contains continuous waveforms, multi-parameter trends, nursing progress notes, medication records and laboratory data from over 3400 patients throughout their ICU stay. When de-identified and annotated, a subset of the data will be made freely available to the research community via our NCRR-funded resource at www.physionet.org. Innovative and sophisticated algorithms and clinician interfaces will be developed to assist in the annotation effort and to create a prototype advanced monitoring system. Evaluation of the new tools and displays will begin in the laboratory utilizing the new database. Later, industry-constructed monitoring system prototypes will be deployed and evaluated in clinical settings at Beth Israel Deaconess Medical Center.

STATUS OF RESEARCH AND PARTNERSHIP

During the second year of the project significant progress has been made in three major areas:

Data. We have expanded our database to include clinical data from over 17,000 patients from medical, cardiac, and surgical ICUs. Continuous bedside monitoring data is included for over 3,000 of these patients. The data occupies almost one terabyte of storage (the MIMIC II Database). Using a sophisticated in-house multi-monitor annotation system, expert clinicians are beginning to identify significant physiologic events (e.g. pulmonary edema, hemorrhage, cardiogenic shock), including causal links to relevant supporting evidence in nursing notes, labs, therapies, trends or waveforms. Annotations and supporting evidence is semi-automatically coded using the UMLS/SNOMED-CT vocabulary. We have completed a first-stage automated de-identification algorithm to remove protected health information from discharge and progress notes. We have also constructed a fully annotated, de-identified and artificially re-identified corpus of nursing notes which will be made available to the public as a "gold standard" for developing and evaluating de-identification algorithms.

Models. Effort is directed at implementing model-based approaches to evaluation, filtering and integration of ICU data, tracking of patient state, and reasoning about patient condition. The static and dynamic models involved are embodied in computer simulations. We have extended our cardiovascular simulations in C and Matlab to include baroreceptor control and an interstitial compartment. Recent work has successfully fitted this model to both simulated waveform data and to real data from a patient with hemorrhagic shock.

Reasoning. Progress has been made on extracting medically relevant meaning from unstructured English text. We have now developed a method for automatically extracting and coding (using the UMLS/SNOMED-CT vocabulary) problem lists from discharge summaries, and are currently in the process of evaluating the system's performance. Progress on information extraction from nursing notes (which are highly telegraphic, information-packed and idiosyncratic notes written during clinical care by ICU nurses) has been directed towards the use of context sensitive tree-search algorithms and statistical learning algorithms (such as hidden Markov models).

The Partnership. The partnership continues to function smoothly. We have expanded our interactions with the clinicians at the Beth Israel Deaconess Medical Center (BIDMC) to begin data-mining of our ICU database to address important clinical questions such as the relationship between ventilator settings and the development of ARDS. Work at MIT, which includes data archiving and analysis (Prof. Mark), modeling (Prof. Verghese), and reasoning/expert systems (Prof Szolovits) continues to move forward. The Philips Company is continuing to support data collection by contributing new hardware and custom software without which we could have no access to the data, and their engineers in Andover and in Briarcliff Manor, NY participate with us as research colleagues.

ISSUES

We have entered a formal relationship with the University of Massachusetts Medical Center to begin new data collection from their pediatric ICU, and have begun the installation of the recording equipment at that site. We have made a de-identified subset of our database available to interested colleagues (from the password-protected site www.physionet.org/database/mimic2db).

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PROJECT TITLE: High Frequency Ultrasound Arrays for Intracardiac Imaging (5-R01-HL067647-04)

PARTNERS' NAMES AND AFFILIATIONS:

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT

Among the most prolonged and detailed interventional catheterization procedures, electrophysiological (EP) mapping and radiofrequency (RF) ablation for atrial and ventricular arrhythmias have received recent attention because of the now well recognized need for spatial mapping in addition to fluoroscopic catheter localization. The complexity and numbers of the procedures being undertaken is increasing. We proposed to design, develop and test a family of 2D and real-time 3D intracardiac ultrasound imaging devices which, at 10-15MHz operating frequency, will provide spatial localization, and both tissue velocity and strain rate estimates of mechanical activation in atrial and ventricular walls, to guide electrical mapping and ablation and resynchronization. Our devices are integrated with EP testing and RF ablation electrodes so that they can visualize the lesion, anatomically monitor the ablation procedure and map the distribution of temperature during RF delivery.

STATUS OF RESEARCH AND PARTNERSHIP

Our partnership has designed and is building three intracardiac imaging devices. The first device, the 64-element EP-enabled hockey stick, (HS) has been tested in porcine models of pacing and ablation and has been found to provide high quality RF resistant imaging of intracardiac anatomy. The IDE application for this device is to be submitted this summer and has required an intense building program of partially and completely fabricated HS.

The two forward-looking devices, so-called microlinear arrays, are in preparation. The piezoelectric device is almost complete and will undergo animal testing this summer. The second, C-MUT version of that device is also being built. The first 9Fr C-MUT-based ring array has been completed, although not mounted on a catheter device. Initial images are encouraging, related to the resolution of this system. Of major importance, computations continue to suggest that the C-MUT technology we are developing, especially in the ring array, with reduced demand for bandwidth, the 64-element, 0.1x0.1mm in size, a power density at the face of the elements of 540W/cm2 corresponding to an output pressure of 4MP should allow the delivery of 3.5Watts focused ultrasound in the focal zone. Thus, we believe we can deliver HIFU as an alternative method for ablation as well as RF energy as originally planned. The ability to provide two ablation methods targeted with the same device used for intracardiac ultrasound imaging would be very advantageous.

ISSUES

Fiscal Issues. The preparation of all of the HS devices needed for the IDE has required the assembly of multiple devices for sterilization testing, accelerated aging materials testing, biocompatibility materials testing, acoustic output characterization and leakage testing. In total, 12 fully functioning devices have been built, plus 69 quasi- and non-functional devices at a cost to the Partnership of approximately $120,000 in effort and materials and an additional $36,000 paid to Precision Interconnect/Tyco for cabling assemblies. This has expended the carryover from the original years of the grant and has all our partners on a tight budget.

Science and Organizational Issues. Two Clinically oriented papers were presented at the American Heart Association meetings in 2004, two at the American College of Cardiology meeting in 2005 and one at the American Society of Echocardiography Scientific Sessions in June 2005. A technical paper was presented at the IEEE meetings in the summer of 2004 and was published in IEEE transactions(ref). A manuscript on the results of animal testing of the HS devices was submitted to the Journal of the American Society of Echocardiography, and a paper on the C-MUT ring array has been submitted to IEEE Transactions on Ultrasonics and Ferroelectrics (UFFC). Lastly, an intense dialogue with the Electrophysiology Division of GE Healthcare has developed, and exploratory efforts are underway to test the possibility of localizing our devices in 3D space with non-contact mapping systems as well as testing their compatibility for guidance with MRI imaging in a collaborative project conjunction with the NHLBI, Division of Intramural Research, Cardiac MRI research group; Robert Lederman, M.D. and Elliot McVeigh, Ph.D.

A late summer meeting of our BRP group and Advisory Board is once again scheduled in the aftermath of the BRP meeting in Washington, D.C.

Ref. D. N. Stephens, K. K. Shung, J. Cannata, J. Z. Zhao, R. Chia, H. Nguyen, K. Thomenius, A. Dentinger, D. G. Wildes, X. Chen, M. O'Donnell, R. I. Lowe, J. Pemberton, G. H. Burch, D. J. Sahn, "Clinical Application and Technical Challenges for Intracardiac Ultrasound Imaging," in Proc. IEEE Ultrason. Symp. 2004, pp. 772 - 777.

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PROJECT TITLE: High Speed Depth Resolved Images of Cardiac Electrophysiology

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT
Development of a high-speed imaging microscope and new fluorescent dyes for monitoring electrical activity and calcium transients deep in heart muscle.

Purpose: We have constructed an optical instrument to map electrical activity of the heart in 3-dimensions and have developed new fluorescent probes this activity. Key focus of the project is on moving into the near infrared so that electrical activity deep in heart tissue can be monitored. This is important for understanding mechanisms of heart disease.

Methods and results: In the instrumentation development part of the program, Drs. Salama, Choi and Lanni tested several camera systems and purchased a unique CMOS camera that scans at 10K frames/s, at 100x100 pixels with a large sensor (1x1 cm^2), low dark current noise and deep electron wells. We have used the CMOS to record action potentials (APs) from the surface of perfused hearts at high temporal (100 μs) and spatial (100x100 μm^2) resolution, yielding APs with 40/1 S/N ratio. Software has been developed to map activation and repolarization patterns for hearts under sinus rhythm, pacing protocols and during fibrillation. Maximizing the S/N ratio and learning how to trigger image acquisition were important to build the 3-D imager based on the CMOS camera and an oscillating Ronchi grating. With Dr. F. Lanni, we have build the first prototype of a 3-D imager for large fields of view (2x2 cm^2) that can resolve images 3-5 mm deep in the myocardial wall (see photo below).

Two functioning prototype imagers were constructed, the first from component parts, and the second by modification of an Olympus DSU confocal scanning optical system. Both units are slit-scan confocal imagers, in which a focused image of a narrow slit in an opaque mask is projected into the specimen. By moving the mask transversely, the slit image is swept across the field-of-view to provide uniform illumination of the scan plane in the specimen. In practice, the slits are arranged radially or otherwise on a disk-shaped mask, which is rotated at high rpm to effect scanning. Fluorescence from the scan plane is imaged back onto the slit, which is followed by a fluorescence bandpass filter. Light passing the slit and filter is re-imaged onto a CCD camera or other detector. Fluorescence originating in out-of-focus zones of the specimen is not well-focused on the slit, and is therefore attenuated relative to fluorescence from the in-focus plane. In general, the axial response of this type of line-scan system is less sharp than for a pinhole-type confocal scanner, but has a significant speed advantage.

Another critical aspect of 3-D imaging of electrical activity is the design and synthesis of new optical probes of membrane potential that have longer wavelength characteristics in their excitation and emission spectra. The goal is to synthesize voltage-sensitive dyes that have greater sensitivity to changes in membrane potential (higher ΔF/F ratio per AP) and function at longer wavelengths to improve depth of penetration of light and reduce light scattering by the tissue. Our partners, Drs. A. Waggoner and L. Ernst have made a set of 5 new probes that can be excited at ~ 700 nm and emit at ~ 850 nm. Two of these dyes, Pittsburgh 1 and 6 (PGH1 and PGH6) has been found to have twice the sensitivity to voltage compared to the best currently available probe (di4-ANEPPS) and can be excited at 690 nm with a peak emission at 850 nm. Optically recorded APs were found to be stable over several hours and to exhibit the

shape and time course of APs recorded with intracellular microelectrodes. High speed spectral measurements of the 'Action Spectra" (voltage-dependent spectral changes) were measured with a linear CCD array that records a spectrum in 20 ms was used to optimize the optical components needed to map APs in 3-D. New dyes are currently being tested under different staining conditions by varying the vehicle to maximize the voltage signals that can be obtained at long wavelengths.

Conclusions: The high speed CMOS camera will allow us to achieve our goals of depth resolved images at depths (5 mm) and speeds (> 1K frames/s) to map impulse propagation from the conduction system of the heart to the myocardium and from the endocardium to epicardium. New fluorescent probes and delivery systems have progressed well with Pittsburgh 1 as the best current probe of electrical acivity in the heart. CMOS images of hearts during fibrillation were of excellent quality allowing us to map the creation and annihilation of reentrant circuits and to map the distribution of sites responsible for the creation of new daughter waves. Rigorous analysis of wavebreak sites indicate that their locations are random and that they do not preferentially occur over large coronary vessels on the heart.

STATUS OF RESEARCH AND PARTNERSHIP

The partnership has worked well. The three research groups come together each month for face-to-face discussions of overall progress and detailed technical problems. The project is on schedule.

ISSUES

None at the present time.

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PROJECT TITLE: Integrated Control of Vascular Pattern Formation

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT
This Bioengineering Research Partnership assembles a team led by two biomedical engineers and a molecular physiologist to focus on the integrative control of vascular pattern formation. While vascular assembly and pattern formation will be needed as critical elements of successful therapeutic collateralization of progressively ischemic organs and in tissue engineering of various tissue substitutes in the future, remarkably little is known of the cells involved, the array of signal molecules and their genetic regulation, and the biophysical factors regulating the spatial and temporal dynamics of vascular pattern formation. Key questions now are: what is the origin of cells responsible for the investment of arterioles with contractile cells and what are the signals that control their proliferation, migration, and differentiation? An integrative systems approach is proposed to measure the dynamics of arteriolar pattern formation in vivo across time scales from the embryo to the adult, and spanning spatial scales from genes to cells to whole networks, and to create a new generation of computational approaches to understand the complex interplay of multiple interacting cells and signal molecules. The specific aims are 1) to determine the role of PDGF and TGF-beta in arteriolar pattern formation during embryonic development, 2) to determine the cell types involved, role of PDGF and TGF-beta signaling, and spatial and temporal patterns of arteriolar assembly in adults, and 3) to develop and use a new cell-based computer simulation to perform integrative spatio-temporal analysis of the arterialization process in the embryo and adult, including multi-signal control of fibroblast and smooth muscle cell proliferation, migration, and differentiation. The multidisciplinary team will utilize unique gene-targeted mice in conjunction with innovative in vivo measurements, and integration of the data into the new computational models will improve understanding of the gene circuitry regulating arteriolar pattern formation. The long term goal is to define the mechanisms that control arteriolar pattern formation, and to provide the basis for powerful therapeutic vascularization procedures that function in the native environment in vivo.

STATUS OF RESEARCH AND PARTNERSHIP
The rational design of therapeutic vascularization procedures requires the ability to understand and control multiple signals regulating normal patterning events. Bone marrow derived stem cells may play a different role in microvascular patterning—mainly a pericytic and signaling role—than has been reported for large arteries. A novel marker of arterial-venous phenotype, NG2, has been discovered and described in networks of two tissues. The role of ECM growth factor modulation has been quantitatively modeled. The computational model demonstrates for the first time that a cell-based simulation can independently predict vascular assembly events, and this provides a broad technology platform for the design of therapeutic vascularization techniques.In the computational automata modeling study of arterialization guided by growth factors or hemodynamic stresses, we were able to predict both new capillary development lengths and arteriolar development quantitatively.

A major focus during the past year has been to determine the contribution of bone marrow derived stem cells (BMS) to arteriolar remodeling in response to systemic hypoxia. In brief, we developed a model of hypoxia-mediated angiogenesis in the mouse spinotrapezius muscle that allows whole mount analysis so that the true shape and location of large numbers of BMC within the vascular network may be observed, along with differentiation markers. C57Bl6 mice were lethally irradiated and reconstituted with bone marrow cells derived from mice containing a EGFP lineage tracing gene knocked into the constitutively active ROSA gene locus. We then exposed bone marrow transplant chimeric mice to systemic hypoxia. In addition, a subset of these chimeric mice were treated with Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) to increase release of circulating BMC. Exposure to hypoxia caused a 13% increase in capillary density relative to control. Hypoxia did not increase the overall number of muscle resident BMC, but did increase the number of rounded BMC by 25%. Of major significance, despite examination of over 10,000 BMC, we found no evidence that these cells contributed to formation of bonafide endothelial or smooth muscle cells. However, some BMC assumed a pericyte-like morphology around capillaries, although they did not stain with antibodies to definitive SMC markers such as SM MHC. GM-CSF treatment further increased the number of round BMC within the muscle and caused a 23% increase in angiogenesis. The results of these studies are of major significance in that they indicate that BMC are not a source of vascular cells during hypoxia-induced arteriolar remodeling. We hypothesize that these BMC play an important role in hypoxia-induced arteriolar remodeling by paracrine release of growth factors.

We have also initiated studies to test the the hypothesis that highly metastatic tumor cells produce soluble factors, including PDGF-BB, that repress the differentiation of vascular SMCs. Consistent with this hypothesis, initial studies have shown that treatment of rat aortic SMCs with media conditioned by highly metastatic C42B4 human prostate tumor cells resulted in profound SMC phenotypic switching including a 67% reduction in expression of the definitive SMC differentiation marker gene smooth muscle myosin heavy chain (SMMHC). Of major interest, we also found that the activity of this unknown factor was not inhibited by an anti-PDGF BB antibody or by treatment with a specific PDGF receptor antagonist. As such, these results indicate that we have discovered a novel factor that is produced by highly metastatic tumor cells that profoundly inhibits differentiation of vascular SMC.

We used the mouse window chamber model to study the influence of disrupted TGF$ signaling on arteriolar and venular remodeling. To this end, we made biodegradable polylactide-co-glycolide (PLGA) polymer particles carrying either TGF$ or a neutralizing antibody to TGF$ (i.e., anti-TGF$). These studies are nearing completion. We also began examining the feasibility of using the mouse window chamber model in conjunction with a new SM-MHC/EGFP transgenic mouse from the Owens laboratory.

ISSUES

Operational issues in the partnership have evolved as the project has matured. Early on, we found that regular joint lab meetings were very important, because new results in one aspect of the team, for example a new cell investment result in a hypoxia model, could affect work being done by another member of the team quite rapidly. The new stem cell projects arose in a very rapid way out of team meetings on this subject area. In our case, the face to face exchanges of ideas and examination of data has been essential. One reason is that the team is addressing issues ranging from tissue level questions and computer modeling to molecular genetic regulation, and the cross-training of students and young scientists has been very effective with the local mix of people bringing different perspectives to the same problems. The aspect that has changed most, and in a positive way, is that these interactions now do not require as many formal lab meeting exchanges as earlier, but post-docs and graduate student trainees simply interchange ideas daily as they work together, and faculty partners interchange ideas in the same way. We have bridged the cultural gap between bioengineering and cardiovascular science labs quite well so that each member of the partnership can easily communicate.

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PROJECT TITLE: MagScrew TAH Testing Through Preclinical Readiness

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT

The fundamental goal of the proposed program is to bring to the point of clinical readiness a new, electrically powered, totally implantable TAH, based on the MagScrew actuator and the biolized blood pump. The specific aims to meet this goal are: (1) To design and develop an advanced technology, fail safe, electronic control unit (ECU), which will maintain the patient's life after an electrical failure, until maintenance is performed. The ECU also contains hardware and patient monitoring capability, and a telemetry function. (2) To build and test refined versions of the remaining system components, based on current state of the art technology. (3) To integrate the components into a functional, complete system. (4) To perform in-vivo performance tests, exercising system capabilities. (5) To perform in-vivo durability tests. (6) To perform bench endurance tests. (7) To complete this work in compliance with FDA Design Controls Regulations.

STATUS OF RESEARCH AND PARTNERSHIP

The current award is in its fourth and last year. A renewal application has been submitted, reviewed and resubmitted. The complete, implantable system has been designed and developed. Some calf implants were performed during the development stage of the program, and the demonstration in-vivo program is underway. Long term implants of 83 and 92 days duration with the complete system have been performed. Two implants are underway, and as of this writing are at 65 days and 17 days post-op. Several more implants will be performed with the available funding.

A bench endurance test is underway, and is approaching a year of continuous running on a full system. A second blood pumping unit has also accumulated significant hours.

The major development and test effort has identified opportunities to increase the reliability of the system. The internal battery cells have worked extremely well, but the associated circuitry has given some troubles. Technology now exists to redesign this circuitry to be more robust. The TETS oscillator circuit can also be redesigned to increase efficiency, while eliminating a component that has sometimes failed. The wiring harness has been satisfactory, but a new partner has been identified who has technology which will further improve this element of the system. The pending grant allows for introduction of product improvements, followed by the formal qualification testing.

ISSUES

Our research is about on schedule. Some tasks are a little behind, but an originally unplanned system size reduction has been successfully implemented, improving the long term value of the technology. The partnership has worked well, and the team has collaborated on new projects stemming from this first collaboration. Perhaps the most challenging issues have been quality assurance of such a hardware intensive project carried out at so many locations, and communication among partners, especially during the early stages when subsystem design and performance was still in a high stage of flux.

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PROJECT TITLE: Biomedical Applications of Electroactive Polymers

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT
    The objective of the Bioengineering Research Partnership program is to refine materials and establish methods for application of electroactive polymers in prosthetics and interventional medical devices. The electroactive materials of interest to us are those that undergo substantial shape change when exposed to an electric field. They are attractive as actuators because of their high energy density – the amount of energy that can be imparted to a load for a given volume or mass of active material, the magnitude of the strain response to an applied field, and their flexibility and toughness when compared with more common electroactive ceramics. Both "found" materials and materials developed expressly for electromechanical activity have been shown exhibit strains of five to 50 percent or more and elastic energy densities on the order of one Joule per cc.
    Two target application areas have been chosen: (1) next-generation prosthetic blood pumps for treatment of end-stage heart disease, and (2) robotic manipulators for minimally invasive surgery, particularly for use in confined spaces such as the thorax. These disparate applications share the need for very compact, efficient and uncomplicated means of actuation. Both suffer today from the need for bulky actuation mechanisms that must remain physically distinct from the parts which pump blood or manipulate tissue. The technology to be developed under this program will blur the lines between structure and actuator, leading to modes of therapy that are not currently available.
    The Materials Research partner is working to optimize electroactive polymers for use in the target device. As these materials are fundamentally different from the active materials used by engineers in the past, the Mechanical Engineering partner is working to develop new design methodologies. The Bioengineering partner is developing prototype devices to demonstrate the potential of the technology and lay the ground work for full development of new devices. Device development is staged so that simpler, proof-of-concept designs are built first, followed by more sophisticated designs as materials and design tools are developed.

STATUS OF RESEARCH AND PARTNERSHIP
    Recent aterials development work has focused on the development of new dielectric elastomers. This class of electroactive polymer is promising because of favorable mechanical characteristics, relative ease of analysis, and relative ease of processing. Most materials used to date are silicones or polyacrylates that provide high energy density due to their dielectric strength. We are developing high dielectric constant materials which we expect to provide similar energy densities at lower electric fields. These are being formulated as insulating polymer matrix-dielectric enhancer aggregates. Both two- and three-component systems have been studied, as have both plain aggregates and functionalized approaches where the enhancer is incorporated into the crosslinks of the matrix polymer.
    The mechanical engineering partner has focused upon development of analytical models, also with an emphasis on dielectric elastomers. Models have been developed for circular thin film membrane and

annulus geometries. Because of the large strains involved, large displacement nonlinear models are required. Data acquired from prototypes of the forms being investigated appear to be as at least as useful in determination of material parameters as is standard large strain tensile testing.

The bioengineering partner has concentrated upon testing of proof-of-concept prototypes and investigation of different forms of actuators that will take best advantage of material properties and processing requirements. Mockup pumps having circular dielectric elastomeric diaphragms have been demonstrated. Diapragms vary in compliance according to the applied electric field. In a manner reminiscent of the action of the natural ventricle, pumping occurs spontaneously upon cyclic application and removal of an electric charge.

ISSUES

The partnership is operating effectively. We rely most upon electronic communications. Allowing graduate students from the different groups the freedom to consult and collaborate has been particularly helpful. Effective partners are motivated chiefly by the desire to work on new problems in a collaborative area.

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PROJECT TITLE: Dynamic Properties of Microbial Adhesins

PARTNERS' NAMES AND AFFILIATIONS:

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GRANTING NIH INSTITUTE/CENTER: National Institute of Allergy and Infectious Diseases

ABSTRACT
  Purpose: The main goal of the proposal is to develop a comprehensive structural picture of how mechanical force affects the functional state of microbial adhesins. A growing number of experimental observations indicate that mechanical forces acting on adhesins may modulate the affinity and selectivity of adhesins to their ligands should give rise to major concern among bioengineers and in the medical profession.
  Methods: In order to test the extent to which mechanical forces may alter the structure and thus the functional states of adhesins, we proposed to characterize the dynamic properties of the most common type of bacterial adhesin - FimH – which is a lectin-like adhesive subunit of type 1 (mannose-sensitive) fimbria of enterobacteria and vibrio. To understand functional changes of the FimH adhesin under tensile force, we are using Parallel Plate Flow Chambers, Dynamic Imaging, Yeast-Two Hybrid system, Steered Molecular Dynamics Simulations, Atomic Force Microscopy, and x-Ray crystallography.
  Results: We have identified distinct structural variants of the Escherichia coli FimH adhesin where shear-flow can induce their preferential binding to target cells, by switching their specificity to the mono-mannoside receptors. We have also conducted SMD simulations in which tension is applied between the receptor-binding residues and the C-terminal end of the self-complemented FimH to develop structural model of how mechanical forces acting on the binding site may affect the tertiary structure of FimH. This model has been tested experimentally and proven to be correct.
  Conclusion: FimH adhesin exhibit behavior of catch bond, strength of which is enhanced by tensile force.

STATUS OF RESEARCH AND PARTNERSHIP
  We are entering final year of the project, with one patent filed and nine manuscripts published, accepted or submitted. Currently, we are preparing a competing renewal of the project, essentially with the same partners.

ISSUES
  The main challenge we have encountered is trying to publish manuscripts with heavy bioengineering component in mainstream molecular biology journals.

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PROJECT TITLE: Regenerative Scaffold Technologies for CNS and Diabetes

PARTNERS' NAMES AND AFFILIATIONS:

Annelise E. Barron, Department of Chemical Engineering; Dixon B. Kaufman, Department of Surgery; John A. Kessler, Department of Neurology; William L. Lowe, Jr., Department of Endocrinology; Phillip B. Messersmith, Department of BioMedical Engineering; Lonnie Shea, Departments of Chemical Engineering and Biomedical Engineering; Samuel I. Stupp, Departments of Materials Science and Engineering, Chemistry, and Feinberg School of Medicine: Northwestern University, Institute for BioNanotechnology in Medicine, McCormick School of Engineering and Applied Science, Weinberg College of Arts and Sciences, Feinberg School of Medicine

GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT

Regenerative medicine is one of the great biomedical challenges of this century, seeking to regenerate parts of the human body throughout life lost to trauma, disease, or genetic factors. Real progress will hinge on our ability to combine effectively the frontiers of technology, biology, and clinical medicine to develop regenerative strategies. This Bioengineering Research Partnership (BRP), proposed by a team of seven investigators in the fields of neurology, surgery, endocrinology, materials science, chemistry, biomedical engineering, and chemical engineering, focuses on two specific challenges of great clinical importance, regeneration of the central nervous system (CNS) and cell replacement therapies for diabetic patients. In this application the target of the team is to develop multiple scaffold technologies and use CNS regeneration and pancreatic tissue replacement as their testing ground. The CNS targets include injection of self-assembling molecules and genetically engineered stem cells into the injured spinal cord or brain following stroke, and the diabetes targets include the development of a subcutaneous islet transplant. The four basic technologies are self-assembling nanofibers customizable to bear multiple tissue specific biological epitopes or have programmable delivery of growth factors; microporous biodegradable scaffolds that deliver genes or growth factors and guide cell migration; post-translationally modified recombinant polypeptides with customizable architecture and bioactivity; and enzyme-driven liquid-to-solid transitions of soluble bioactive peptides. The integrated scaffold technologies proposed include, the use of self-assembling nanofiber technology to modify microporous materials and create micro-nano hierarchical scaffolds, the adaptation of recombinant polypeptides for in situ enzyme driven solidification, and the development of bioactive two-phase molecular composite scaffolds containing linear polypeptides and peptide nanostructures.

STATUS OF RESEARCH AND PARTNERSHIP

Work in the past funding period—the first year on this award—produced accomplishments in technology development for both clinical target areas outlined in the proposal: regeneration of the central nervous system (CNS) and cell replacement therapies for diabetic patients. Our efforts are highly collaborative and integrated, but to crystalize initial successes they are attributed here to the contributing laboratories:

In Stupp's laboratory islets were placed on poly (L-lactic acid) (PLLA) scaffolds coated with peptide amphiphile (PA) gels before implantation. This scaffolding localizes islets to the transplant site and improves retention and survival. The scaffolds have been found to significantly improve islet viability and function. For spinal cord repair efforts have focused on designing PA molecules for in vitro and in vivo

imaging and tracking, including fluorophore-containing PAs and magnetic resonance (MR) contrast agents, and on modifying IKVAV PA molecules to control gelation kinetics under injection conditions.

In Messersmith's laboratory In vitro islet survival was evaluated morphologically by fluorescein diacetate and propidium iodine staining, while islet function was tested using insulin content radioimmunoassays and glucose challenge radioimmunoassays. Islets derived from FVB mice cultured within the hydrogels demonstrated no significant gain or decrement of survival or function compared to non-encapsulated controls. For in vivo studies an in situ gelation strategy for encapsulating murine islets proximal to the epididymal fat pad was developed. In collaboration with the Kaufman group, efficacy of islet transplant at this less-invasive site was demonstrated.

Kaufman's laboratory provided the surgical expertise to do islet implants and evaluate the newly developed technologies. Using diabetic mice, testing prefabricated foam, microporous and micro-nano hierarchical scaffolds to enhance the efficacy of islet transplants is under way.

Shea's laboratory has made traditional porous sponges, cylinders, and scaffolds with multiple channels. Protein incorporation into these scaffolds exhibited a sustained release for at least 42 days, with the release rate controlled by the method of incorporation and the polymer molecular weight. Tissue engineering scaffolds capable of sustained plasmid release were examined to promote gene transfer locally and stimulate new tissue formation. Wet granulation was shown to enhance in vivo transgene expression, possibly through the increased loading and maintenance of the scaffold pore structure.

Lowe's laboratory worked towards transplanting islets on microporous scaffolds into abdominal fat. These islets have continued to function for more than 180 days. Of note, islet function following transplantation on scaffolds into abdominal fat was significantly better than that of islets transplanted free into abdominal fat, as defined by the days to euglycemia and glucose levels during an intraperitoneal glucose tolerance test.

In Barron's laboratory genetic engineering was used to create high-molecular weight protein polymers that will serve as substrates for enzymatic cross-linking, for in vitro or in vivo liquid-to-solid transformations using only biomolecular chemistry. The resulting target proteins allow for chemical grafting of synthetic, bioactive peptides.

Kessler's laboratory has achieved substantial progress in the study of the effects of self-assembling peptide amphiphiles on recovery from spinal cord injury. Injecting the self-assembling material was found to significantly reduce formation of the glial scar, as evidenced by a reduction in immunostaining for glial fibrillary acidic protein (GFAP) and a reduction in chondroitin sulphate proteoglycans. Further, injection of the gel resulted in significant functional improvement. An initial examination of dorsal column (proprioceptive) tracts showed that some fibers entered and traversed the damaged area of spinal cord in amphiphile-injected animals whereas no such fibers have yet been seen in animals injected with control material. In toto these observations indicate that injection of the IKVAV peptide amphiphile results in a diminution of glial scar formation and an increase in functional recovery after spinal cord injury.

In Barron's laboratory genetic engineering was used to create high-molecular weight protein polymers that will serve as substrates for enzymatic cross-linking, for in vitro or in vivo liquid-to-solid transformations using only biomolecular chemistry. The resulting target proteins allow for chemical grafting of synthetic, bioactive peptides.

ISSUES

After the initial start-up period no significant issues have been encountered and research is progressing as expected.

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PROJECT TITLE: Bioengineering Research Partnership for Muscular Dystrophies and Cell Therapies

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GRANTING NIH INSTITUTE/CENTER: National Institute of Arthritis and Musculoskeletal and Skin Diseases

ABSTRACT

Anchorage-dependent cells exert tractions on their matrices, allowing them to feel and respond to matrix elasticity through myosin-based mechanisms. Mesenchymal stem cells (MSCs) are anchorage-dependent and, we show, have elasticity-directed differentiation on matrices typical of tissues. Under constant serum conditions and low cell density, respective commitment toward neuroblasts, myoblasts, and osteoblasts is seen on soft matrices typical of brain, stiff substrates typical of muscle, and very stiff substrates likely to be typical of collagen-I coated bone. However, incomplete expression via matrix stiffness alone, relative to control cells, is augmented by stimulation with growth factors thus inducing full lineage commitment; chemical or physical stimulus alone cannot. During differentiation, myosin-IIa remains relatively constant while organizing into striations only on matrices mimicking muscle elasticity. Myosin-IIb, however, organizes peripherally and increases with substrate stiffness as well as focal adhesion size and number, implying an adhesion-contractility-based differentiation mechanism. Indeed, contractile inhibition of myosin-II or Rho GTPases abrogates the elasticity-directed differentiation response. Physical measures of cell tension further demonstrate increased cell contractility with increasing matrix stiffness and a relaxation upon myosin inhibition, consistent with mechano-sensing by stem cells. When these stem cells are used in a therapeutic manner to combat fibrotic diseases including muscular dystrophy and myocardial infarction, even though these stem cells are surrounded by proper chemical signaling, differentiation is limited by the surrounding stiff microenvironment, indicating its possible overriding importance during selected differentiation programs.

STATUS OF RESEARCH AND PARTNERSHIP

This BRP, in its final year of initial funding, has elucidated the functions of several key regions of the protein dystrophin as well as how adhesion and contractility within a cell physically regulate a muscle or other adherent cell's overall differentiation state, a process which is mechanotransduced through dystrophin. This BRP has greatly benefited from other external collaborations at the University of Pennsylvania, namely the Institute of Medicine and Engineering and Pennsylvania Muscle Institute.

ISSUES

Remaining questions include exactly which portions of dystrophin are critical to maintain function and transmit contractile force within a muscle cell. Recent use of "exon skipping" techniques, which deliver anti-sense oligonucleotides to cells in order to omit mutated regions, provide a practical clinical application of our studies. Knowing the mechanically and structurally important portions of the protein will help design better and more feasible 'skipping.' Clinical outcomes are also bolstered by cell-based techniques investigated here, but remaining issues include cell delivery, integration, and a proper matrix in which the cells become resident.

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PROJECT TITLE: Complex Nanocomposites for Bone Regeneration

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GRANTING NIH INSTITUTE/CENTER: National Institute of Dental and Craniofacial Research

ABSTRACT
Our BRP program is aimed at development and testing of new implant materials by combining biomimetics with two radically new design philosophies to produce dense and strong bioactive scaffolds that are intended to be partially or completely resorbed and replaced by bone from the host in a sequence resembling bone remodeling. Three types of materials will be developed. First, inorganic scaffolds with a dense core and a graded distribution of porosity and surface chemistry will be fabricated by stereolithography and by a novel technology developed in our laboratory based on freeze casting of calcium phosphate suspensions. Second, hydrogels and self-assembling polymers that possess anionic groups and adhesive ligands suitably positioned for the nucleation process and cellular adhesion will be used to direct template-driven biomimetic mineralization of hydroxyapatite and other biominerals in nanoscopically and microscopically controlled fashion. Third, the resultant porous scaffolds will be used as the matrices to fabricate inorganic-organic composites with improved strength and fracture resistance. This will be achieved by infiltration of the inorganic scaffolds with hydrogels or by direct template-driven biomimetic mineralization of calcium phosphate nanoparticles on the organic scaffolds. Materials that pass the mechanical property tests will be tested in cell cultures and an animal model.

STATUS OF RESEARCH AND PARTNERSHIP
The second year of the project has been very productive with significant progress having been made on most of the specific aims. During the second year we focused on four areas: (1) investigation of bone fracture; (2) we developed new techniques for the preparation of porous inorganic materials; (3) we discovered novel method for the mineralization of polymer scaffolds, and, (4) we discovered new process for preparing flexible pHEMA-HA composites with mineral-to-organic matrix ratios approximating that of human bone.

1. Fracture of bone. Our investigations of the fracture behavior revealed that the primary mechanism of toughening in both dentin and cortical bone was the formation of crack bridges - islands of uncracked material in the wake of a growing crack that hold it together and lower the driving force felt by the crack tip. With age, the amount of such bridging was observed to be lowered, leading to a reduction in the toughening which was correlated mainly to changes that are observed at the microstructural size scales. Our studies further revealed changes at the nanostructural level that could also be related to the process of aging. Work on the fatigue (repetitive cyclic loading) behavior of these materials suggested that a "true" cyclic fatigue mechanism involving alternating blunting (from the time-dependent visco-elastic/plastic response) and re-sharpening (from the unloading) of the crack tip, is active. These studies give us a better appreciation for the important role that the organic component of any replacement material will need to perform in order to truly replace bone.
2. Preparation of porous ceramic scaffolds. During the last year, the work has focused on two techniques: infiltration of polymer foams and use of porogens. Diverse ceramic materials have been

employed with particular emphasis on hydroxyapatite (HA), a calcium phosphate closely related to the mineral component of bone. Infiltration of polymer foams. Cellular ceramics have been fabricated through the infiltration of a polymer sponge with a ceramic slurry until the inner polymer walls are completely coated by the ceramic powders. Subsequently, the sample is fired to remove the polymer and form a ceramic skeleton that is strengthened by sintering at high temperature. The cellular ceramics obtained by polymer infiltration exhibit a microstructure formed by round, interconnected alveoli (~100-200 μm wide). The alveoli walls are fully dense and their thickness ranged between 10 to 80 μm. The compression test of a cellular ceramic shows a very diffuse damage with a strong reinforcement. The samples can retain overall integrity up to deformations higher than 30%. Use of porogens. Glassy carbon spheres were used to create porosity on ceramic compacts. Ceramic slurries containing up to 50 vol% of glassy carbon spheres (20-50 μm) were homogenized and dried. The powders were pressed at 1000 MPa and the compacts fired. The carbon spheres burn away during firing leaving open porosity. The porous samples thus fabricated using porogenes show a round porosity similar to those obtained by the infiltration of polymer foams.

3. Preparation and characterization of poly-lactic/HA composites. PolyL-lactic acid (PLA) is a bioresorbable polymer currently used in many orthopedic applications. HA particles can be used to reinforce PLA and to buffer the intermediate acidic products that result from the polymer degradation in vivo that often lead to adverse inflammatory response. During this year we have developed a simple processing route for the fabrication of PLA-HA composites and we have initiated the study of their in vitro degradation from a micromechanistic perspective. Composites with ceramic contents ranging between 75 to 85 wt% have mechanical properties that match those of human cortical bone: elastic modulus of ~10 GPa, strengths of ~ 60 to 130 MPa and toughness values of ~ 100 to 275 J/m2, as compared to lower-bound values of 10 GPa, 35 MPa and 100 J/m2 for human cortical bone. However, the properties deteriorate with immersion in Hanks' Balanced Salt Solution (HBSS) with such degradation being more pronounced for samples with larger hydroxyapatite contents. Indeed, strengths and toughness values of the composites with 80 and 85 wt% HA deteriorated to values lower than that of bone over the period studied (up to 4 weeks). The decrease of the mechanical properties was accompanied by a definite degradation of the underlying microstructure with definitive evidence of dissolution of the polymer matrix between the ceramic particles.

4. Mineralization of hydrogel scaffolds. The controlled integration of organic and inorganic components confers natural bone with superior mechanical properties. Bone biogenesis is thought to occur by templated mineralization of hard apatite crystals by an elastic protein scaffold, a process we sought to emulate with synthetic biomimetic hydrogel polymers. Cross-linked polymethacrylamide and polymethacrylate hydrogels were functionalized with mineral-binding ligands and used to template the formation of hydroxyapatite. Strong adhesion between the organic and inorganic materials was achieved for hydrogels functionalized with either carboxylate or hydroxyl ligands. The mineral-nucleating potential of hydroxyl groups identified here broadens the design parameters for synthetic bone-like composites and suggests a potential role for hydroxylated collagen proteins in bone mineralization. Human osteoblastic cells were found to attach, spread, and proliferate on all synthetic hydrogel copolymers tested with no apparent cytotoxicity.

The hydroxylated hydrogel was also explored for the preparation of high HA content flexible 3-D composites. A fast and convenient process for preparing flexible pHEMA-HA composites with mineral-to-organic matrix ratios approximating that of human bone was discovered. A wide range of hydrogel formulation and processing conditions, along with the variation in the crystallinity, particle size and aggregation properties of HA, are used for the fabrication of bulk composites. The correlation between the mechanical properties of the resulting composites and these parameters are being investigated. Optimized flexible pHEMA-HA composites generated using this approach will be further evaluated for in vivo performance using animal models as potential bone implants.

ISSUES
None.

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PROJECT TITLE: Cellular Engineering for Metabolic Stasis

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT
     As several living cell-based therapies are approaching clinical utility, emphasis must now be placed on the fundamental and practical issues associated with the translation of these technologies from bench-to-bedside. Although there have been significant advances both at the fundamental and practical levels for scale-up of bioreactors, culture of cells, and implementation of safety and regulatory policies, the tools and understanding needed for the storage of living cells and complex tissue constructs lags significantly behind. In nature, many animals and organisms down-regulate their metabolism and may enter into a state of stasis by either desiccation through removal of water from their cells (i.e., anhydrobiosis) or by a developmentally-programmed arrest under full hydration (i.e., diapause). The ability to enter diapause prior to desiccation is crucial for the survivorship of many organisms that undergo natural states of dormancy. Furthermore, a common theme is that desiccation-tolerant animals accumulate large amounts of disaccharides, especially trehalose and sucrose. These sugars provide protective effects by forming stable sugar glasses at high water contents, and by stabilizing biological membranes and proteins through direct interaction with polar residues. We, therefore, hypothesize that metabolic pre-conditioning of mammalian cells to induce diapause-like state followed by controlled drying conditions can be used to achieve desiccation tolerance in mammalian cells and tissues. To this end, our 3 specific aims are: [1] To develop optimal physicochemical conditions to stabilize desiccated cells; [2] To metabolically precondition mammalian cells to improve survivorship during storage; and [3] To develop metabolic and biophysical strategies to accelerate recovery of desiccated cells.

STATUS OF RESEARCH AND PARTNERSHIP
     Optimization of poration and desiccation conditions. We utilized the mouse macrophages, which are known to express endogenous ATP4- sensitive P2X7 receptor channel, to load the membrane impermeable compound trehalose into the cytoplasm of these cells. For ATP treated cells that were porated with this protocol a nearly linear increase in the intracellular trehalose concentration was observed over time. Cells that were loaded trehalose with the above protocol and dried to different moisture levels (gH2O/gDW) showed superior survival at all moisture levels investigated.
     Detection of the phosphorylation state of AMPK in different cell lines. The AMP-activated protein kinase (AMPK, EC 2.7.1.37.CAMK) is part of an ultra-sensitive system for monitoring cellular energy changes and could be part of the metabolic depression and cell stasis observed in some naturally occurring states of latency and the associated tolerance of severe environments. Two major mechanisms of activating AMPK in vivo can be distinguished from each other. Direct binding of AMP to the enzyme activates AMPK allosterically, whereas modification of the enzyme by the upstream kinase LKB1 increases AMPK activity several fold via phosphorylation at the threonine 172 site. We developed a Western Blot assay that allows us to detect both the phosphorylated (P+) and the total amount of

expressed AMPK (P-) in several cell lines tested. We also developed methods for loading membrane permeable and impermeable activators of AMPK into cells. Thus we can now relate the phosphorylation state of AMPK in vivo to changes in cellular metabolism and cellular desiccation tolerance promoted by AMPK activators like AICAR and AMPS.

Mitochondrial permeability transition. Using DNA microarray technology, we identified that cyclophilin D gene expression is downregulated in the diapause state of A. franciscana. Cyclophilin D is part of a multi-protein complex in the mitochondria membrane (mitochondrial permeability transition pore, MPTP) which is involved in apoptotic and necrotic cell death. The minimum constituents of the regulated MPTP are believed to be, in addition to cyclophilin D, the voltage-dependent anion channel (VDAC) and the adenine nucleotide (ATP/ADP) translocators (ANTs). When mammalian mitochondria are exposed to high calcium concentrations in the presence of the co-activator Pi, a large swelling, uncoupling of respiration and release of cytochrome c, which activates the apoptotic cascade, can be observed. All major components of the MPTP could be detected in mitochondria from A. franciscana. However, if mitochondria from A. franciscana are challenged with calcium concentrations that induce the MPTP in mammalian systems no permeability transition can be observed. This result is especially exciting because recent evidence shows that mammalian mitochondria devoid of ANTs or cyclophilin D exhibit reduced calcium sensitivity, but still undergo the permeability transition at high calcium concentrations. Understanding the mechanism by which A. franciscana avoids the permeability transition may enable us to precondition mammalian cells in ways that improves desiccation tolerance.

Trehalose loading into mitochondria. To test whether trehalose needs to be present in mammalian mitochondrial matrix for a high degree of desiccation tolerance, we introduced trehalose into isolated rat liver mitochondrial matrix by reversibly permeabilizing mitochondrial inner membrane using mitochondrial permeability transition pore (MPTP). The results showed that following drying to similar water content, the mitochondria loaded with trehalose had significantly higher inner membrane integrity than those without trehalose loading. These findings suggest the presence of trehalose in the mitochondrial matrix affords significantly improved desiccation tolerance to the isolated mitochondria.

ISSUES

We plan to start investigating the stability of desiccated mammalian cells in the presence of intracellular trehalose. We will study the physicochemical conditions leading to maximal survivorship including cell interactions with the surface and microscale flow patterns in drying droplets. In tandem with these studies we will continue our investigations with respect to metabolic regulators to induce diapause like state in mammalian cells. We will measure oxygen uptake rate and the heat output to quantify cellular stasis. We will also design a cell repair solution to rehydrate dried cells. This solution will involve both co-polymers to stabilize cell membranes and also various molecular additives to minimize apoptosis and free radical damage. Two manuscripts have been submitted from the work summarized above. First manuscript focuses on the poration and desiccation of mammalian cells using trehalose. Second manuscript relates to the beneficial effect of trehalose loaded into the mitochondrial matrix.

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PROJECT TITLE: Tissue-Engineered Valve From Cell-Remodeled Biopolymer

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT
   This BRP aims to develop a tissue-engineered cardiovascular valve, with the initial focus being an aortic valve replacement. The "tissue-equivalent" approach to fabricating bioartificial tissues, in which a fibrillar biopolymer gel (type I collagen or fibrin) is contracted, aligned, and remodeled by entrapped tissue cells, will be used. A tissue mechanical theory will be applied to determine the optimal mold design such that cell-mediated compaction of the gel around the mold surfaces yields the target geometry and ECM fiber alignment. A coupled solid-fluid mechanical model of valve function in pulsatile flow will be used to define what alignment-dependent mechanical properties of our "valve-equivalent" (VE) are desired following incubation for proper valve function, and to simulate what the VE function will be. Various experimental strategies will be implemented to manipulate these properties during incubation. Measurements of these properties will be used to develop a microstructural constitutive model of the tissue resulting from the cell-remodeled gel that is needed in the model of valve function. High-speed ultrasonic imaging of leaflet motion will be developed and used along with particle imaging velicometry in order to validate the model as well as visualize valve function. In addition to comprehensive biological and biomechanical characterization of the VE, novel adult stem cells will be assessed as a source of endothelial cells and, potentially, interstitial leaflet cells for VE fabrication. An animal study will be performed to access the remodeling that occurs in vivo and its effect on valve function.

STATUS OF RESEARCH AND PARTNERSHIP
   Valve-Equivalent Fabrication. Several advances were made in the fabrication of fibrin-based valve-equivalents (VEs). A major study was performed to compare porcine aortic valve cells (PAVCs) and human dermal fibroblasts (HDFs) as possible cell sources. Their ability to remodel fibrin gel was assessed in a model construct (adherent disk-shaped constructs). Based on collagen production and tensile mechanical properties, neither cell type proved superior (Williams et al.).
   We fabricated a number of VEs with HDFs and analyzed the roots and leaflets for composition and tensile mechanical properties. Alignment of the cell-contracted fibrin and cell-produced ECM that is replacing the fibrin were characterized. Circumferential alignment in the root and commisure-to-commisure alignment in the leaflets was established. The stiffness of the leaflets was ~0.5 MPa with the current conditions, about a factor of 10 below native leaflets.
   Particle Image Velocimetry. For calibration and testing purposes of our 3D PIV system to be used in characterizing flow thru the VE, an apparatus was developed to generate a steady and repeatable recirculating flow through a circular pipe with one inch diameter. The pipe is surrounded by a liquid-filled box and liquid-filled prisms optimized to yield high quality images with minimal distortion. The stereo PIV optics and hardware were tested extensively and optimized with this configuration so that

accurate velocity field measurements can be acquired in planes normal to the flow. It was found that this configuration requires both precise laser sheet alignment and accurate calibration, using a novel algorithm, in order to yield high-quality data. Separate '2D' PIV measurements of planes parallel to the flow have also been acquired. A pulsatile flow system (Vivitro Systems) that can produce both physiological and pathological cardiac rhythms was purchased and tested.

Ultrasound Imaging. We established the use of the Technos MPX's small part transducer in imaging the VE undergoing lateral stretching (e.g. during uniaxial test). We also established the feasibility of characterizing lateral flow of water with fine microspheres for potential characterization of the flow field in the flow loop (Ebbini). Therefore, our real-time 2D imaging system is now ready for use to characterize the VE in the flow loop. Towards a scanning imaging system, we focused on characterizing the components of single-element mechanically scanned system and finalizing the design for the 3D mechanical scanning unit. In addition, we began the investigation of high frequency PVDF receiving arrays to be used in real-time imaging with little or no mechanical scanning. In particular, the following tasks were completed: (1) Segmentation algorithm for 3D valve data to produce a solid for visualization and for use in the computational model was developed. High quality 3D data sets can with full speckle components from the valve (frequencies from 15 40 MHz were used). (2) Mechanical scanning system employing a 1-stage servo motor has been built and was fully tested in image acquisition. The current system employs a rotational stage suitable for sector scans. We will soon be receiving a 2-stage xy servo unit that will allow 3D image acquisition in Cartesian coordinates. (3) Arbitrary function generator has been incorporated in the image acquisition system. Arbitrary waveforms with frequency components up to 50 MHz are possible with this system. (4). 16-element PVDF arrays have been fabricated and characterized along with associated electronics for transmit circuitry.

Computational Valve Mechanics. We completed the 3-D adaptive finite element solution to the Anisotropic Biphasic Theory equations for regular geometries, marking the first time that the model equations have been solved in 3-D. The solution describes the strain-induced alignment observed experimentally by Kolodney and co-workers. We have also set up the mesh generator necessary to perform more complex simulations.

We implemented a 2-D overlapping-grid modeling scheme for embedding a thin structure (i.e., the valve leaflet) in a moving fluid. The method uses one solid grid (the valve) and two fluid grids (near-valve and bulk fluid) to overcome the difficulties associated with the large pressure drop across a thin leaflet and the large range of motion. As an initial study of the distributed Lagrange multiplier methods to be used in valve modeling, we simulated motion of a sphere embedded in a (fixed) fluid grid (Nordsletten et al.).

Significance. We have demonstrated the basic ability to fabricate a fibrin-based VE, to perform 3D PIV, to implement a wrapped-embedding approach for simulating leaflet motion, and to image the VE leaflets and their displacement using ultrasound. We are thus positioned to develop a functional VE that may ultimately provide a preferred alternative to mechanical and bioprosthetic heart valves, especially for juvenile patients.

ISSUES

There are no issues regarding the partnership; frequent interactions and a high degree of coordination result from all the investigators being at the University of Minnesota.

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PROJECT TITLE: Intracortical Visual Prosthesis

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT

The development of an implantable human cortical visual prosthesis has been a goal of neuroprosthesis research for 30 years. During this time, the NIH has funded intramural and extramural studies to advance fundamental technologies and address biological questions necessary for the design and fabrication of an implantable system to stimulate the primary visual cortex with intracortical microelectrodes. Although previous work addressed portions of these issues, the focus has primarily been on technology, and fundamental questions remain in three critical areas of research:

Physiology: How can we maximize the amount of information transferred to the primate brain through an array of intracortical stimulating electrodes? In particular, what is the optimal manner of delivering stimulus through the electrodes, and how can stimulation through multiple channels be patterned to best control perception?

Electrode Technology: Can intracortical electrodes be designed, fabricated, and implanted, allowing for long-term safe chronic stimulation of the primate visual cortex by large numbers of electrodes?

Implantable Stimulation Hardware: Can reliable modular implantable electronic packages, capable of driving large numbers of electrodes, via transcutaneous RF power and bi-directional data links, and suitable for surgical implantation, be designed and fabricated?

The overall objectives of the Intracortical Visual Prosthesis BRP are to advance the technology sufficiently to provide a reasonable expectation of reliability and safety for implantable hardware, and to develop an animal model to perform crucial psychophysical and electrical stimulation studies. This 4-year project will culminate in an analysis of data from the fundamental electrical stimulation and psychophysical studies of an animal model, and the development of a completely implantable multichannel stimulation system, including chronically implantable stimulation electrodes. Our long-term goal is to develop an implantable system that will provide usable vision for a large population of persons with blindness. The goals of this 4-year project are to answer the fundamental questions, above. Our short-term goals are to: (1) Develop a primate animal model for testing the sensory responses to large numbers of parallel intracortical stimulation electrodes. (2) Extend earlier human work on point-phosphene perception to a more general approach that tries to exploit other V1 tuning properties, such as orientation selectivity, to create a richer visual feature set. This will be done by a combination of recording and stimulation techniques in highly trained monkeys performing psychophysical tasks. (3) Demonstrate safety, efficacy, and electrochemical stability of our proposed intracortical electrode arrays using a combination of in vitro and in vivo testing. (4) Determine the optimal physical configuration for, and design a high-reliability implantable inductively-powered cortical stimulator, interfaced to an external

computer controller. (5) Develop safe implantation methods, including pre- and postoperative imaging techniques, to optimize and minimize the duration of implant surgical procedures.

STATUS OF RESEARCH AND PARTNERSHIP

Progress has been made in all three of the critical areas.

Physiology: Psychophysical testing of our 3rd non-human primate, Garp, is addressing the questions of whether orientation percepts can be used to communicate artificial visual information, and where a collection of orientation percepts can be integrated to form a single perception.

Electrode Technology: Electrode implanted in Garp have demonstrated a significant reduction (90%) in charge capacity over the initial 2-month period. Subsequent measurements have revealed that with daily chronic stimulation some recovery is seen. We hypothesize that a tissue encapsulation mechanism prevents the necessary counter ion availability needed for the charge transfer.

Implantable Hardware: Design on our wireless array has progressed. We have ceramic electrode platforms, and the second generation silicon chip under test.

The participation of the team members and the group dynamics are excellent.

ISSUES

Presently there are two key issues that our team is aggressively devising studies to address: The cause, significance and remedy for the electrode charge reduction, in-vivo, and the qualification of the wireless module design.

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PROJECT TITLE: Particles in the Developing Lung: Bioengineering Approach

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT
This bioengineering interdisciplinary partnership project plans to use engineering expertise to develop a combination of tools, including computational fluid mechanics, the development of particle technology, and physiological approaches in animal models, to be utilized in a comprehensive study on particle deposition, retention, and clearance pathways in the developing lung. There is no more important imperative in our society than to protect the health of children, yet the specific differences in pulmonary structure between neonates, children, and adults have not been considered when assessing health risks associated with environmental exposure to aerosol particulates. Children's lungs postnatally undergo remarkable structural changes, such as a dramatic increase in alveolation, in addition to an increase in size. Our recent studies clearly indicate that the structure of the acinar airways has a profound influence on fine particle deposition. It is, therefore, very likely that particle deposition, retention, and clearance pathways in infants and young children are significantly different from those in adults. In particular, our preliminary data suggest that health risks may rise rapidly postnatally and peak between 2 and 5 years. However, little is known about the qualitative and quantitative aspects of particle deposition in developing lungs, mostly because these questions are not accessible to clinical studies or experimentation for ethical and technical reasons. We propose (1) to establish computational fluid mechanics methods and investigate the effects of structural changes during lung development on deposition; (2) to develop a state-of-the-art high precision lung function/inhalation detection methodology utilizing engineered tracer particles, and (3) to apply this new methodology to investigate how particles are deposited and retained in an animal model utilizing postnatally developing rats. These proposed studies will allow us, for the first time, to get a comprehensive picture of the changes in particle deposition-retention associated with lung development. This knowledge has important implications for the estimation of health hazards posed by particulate air pollution and for the establishment of age-appropriate doses of therapeutic drugs delivered by aerosols.

STATUS OF RESEARCH AND PARTNERSHIP
In the second year of this BRP project, research is progressing as planned. Analytical investigation: We investigate how the interaction of wall motion with alveolar recirculation flow affects transport, and this year, we made a significant step forward. The model we have devised uses Moffatt corner eddies in a steady Stokes flow, which are perturbed by unsteady wall motion. This model is simple, but captures the essential features present in alveolar flow, and it is suitable to examine how changes in different parameters result in qualitative changes in transport. The main aim was to see whether recirculation is persistent under wall perturbation. It was found that, given an eddy with sufficiently strong recirculation, particle paths appear to break up into chaotic orbits. This mechanism enhances the mixing of particles.

Computational investigation: We are also making progress in the computational part of the investigation. Continuing our previous efforts, we are working to develop a finite element computational model of a rhythmically expanding/contracting alveolated duct to study potential effects of developmental changes in acinar architecture on alveolar wall motion.

On the experimental side, we continue working in two areas. (1) We are developing state-of-the-art custom-made apparatus needed for the planned experiments. One of the equipment being developed is a PC-controlled high precision lung function test unit for postnatally developing rats. This year we finalized a unit for adolescent and adult rats by redesigning the "head" of the ventilator. The improved system exhibits an instrumental dead space volume of 300ul, which is only about 24 % of the anatomical dead space of an adult rat. (2) We also apply newly developed methodology to collect data. Lung functions of both spontaneously breathing rats and mechanically ventilated rats were measured at different stages of postnatal lung development. The major finding was that 7-day old rats breathe with tidal volume less than the anatomical dead space at frequency of 250/min spontaneously. Total deposition of inhaled ultrafine particles was also measured. The results show that whereas no appreciable deposition occurred in spontaneously breathing 7-day old rats; particle deposition was detectable in 14-day old rats, and it increased at the age of 21-days.

BRP partnership meetings were held several times. The Harvard-GSF partnership meetings were held on October 6-8, 2004 at the GSF site (Munich) where the PI (Dr. Tsuda) and all investigators/staff members at the GSF site attended the meeting; and on May 22, 2005 at San Diego where the PI and the key personnel of the GSF site (Drs. Kreyling, Schulz and Semmler) exchanged the updated information on research progress of each site. The Harvard-Surrey Partnership meeting was held on October 28-29, 2004 at Harvard University (Boston) where Dr. Laine-Pearson (Surrey) reported her progress to the Harvard group members. To facilitate the newly established Harvard-Kragujevac partnership, Dr. Kojic (an investigator of the Harvard site) is currently in Kragujevac and will work with the investigators in Kragujevac for three months (June-August, 2005).

During the current year, an additional partnership has been established with the Center of Supercomputing at the University of Kragujevac, Serbia (the project leader: Dr. Vlastelica). The partnership with this new site is expected to enhance the power of our computational modeling.

ISSUES
None.

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PROJECT TITLE: High Frequency Nonlinear Acoustic Intravascular Imaging

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT
Intravascular ultrasound (IVUS) imaging is a technology that permits tomographic visualization of a cross section through the vessel wall. Its development has provided a powerful new method to assess plaque morphology in vivo. However, while new catheter designs are markedly improved on their predecessors, image quality has not seen significant gains due to the primitive nature of the ultrasound transducer designs. Increasing the frequency of the transducer above the current state-of-the-art 40 MHz holds the potential to improve image quality, although higher frequencies are attenuated rapidly in biological media and the depth of penetration is therefore reduced. One possible method of enhancing the quality of the IVUS images may be to exploit the effect of nonlinear propagation (harmonic imaging) of the ultrasound signal as it passes through the tissue. Despite the fact that harmonic imaging is now becoming a standard modality in the latest commercial B-mode ultrasound scanners with a frequency range up to 4.0 MHz, there is no evidence of attempts to develop a harmonic imaging system for significantly higher frequencies, which would be suitable for intravascular applications. In this application we propose to investigate the generation of tissue harmonics at fundamental frequencies (20 to 40 MHz) suitable for intravascular application. This will be pioneering work in the field of medical acoustics. The major driving force for our project will be clinical necessity. We envisage that the implementation of high frequency harmonic imaging will dramatically improve image quality and allow better delineation of plaque geometry and composition. High frequency ultrasound transducers will be designed and built comprising traditional ceramic materials and novel polymeric devices fabricated using MEMS technology. Finally advanced signal processing methods will be designed and developed to accurately predict plaque composition from high frequency nonlinear acoustic data.

STATUS OF RESEARCH AND PARTNERSHIP
At this stage we feel that Specific Aim 1 is almost completed to schedule. The final test of the model will be performed using experimental data collected with assistance from Dr. Cheri Deng in Case Western Reserve University. Also, we intend to broaden the frequency range up to 80 MHz as the acoustic probe with corresponding bandwidth is becoming available through Force Technology, Inc. (Denmark). Specific Aim 2 is still consuming the bulk of our efforts. Our custom-manufactured PVDF transducers provide the second harmonic signal at the level -25 to -20 dB. Previously we attempted bandpass filtering to obtain second harmonic signal, however the signal-to-noise ratio was poor due to parasitic capacitance. We will try to overcome the problem. Also, we will investigate the possibility of pulse inversion technique with our PVDF transducer. For this purpose we need to buy additional equipment: random signal generator and amplifier with low harmonic distortion in electronic circuitry.

Pursuing Specific Aim 3 we are developing a theory of impedance analysis and spectral analysis of harmonic data and currently in the process of negotiation with Krautkramer Branson/AGFA Co. to obtain the custom-built by our order bi-frequency piezoceramic annular array transducer for the separation of transmit and receive modes and second harmonic super-resolution inverse scattering harmonic imaging.

ISSUES
No input.

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PROJECT TITLE: Advanced Multi-Spectral Imaging (MSI) System for Medical Diagnostics

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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute and National Institute of Biomedical Imaging and Bioengineering

ABSTRACT

The goal of this project will be to develop a novel multi-spectral imaging (MSI) system using the synchronous luminescence (SL) concept to rapidly detect cancer in-vivo. The proposal will address the problem of real-time in-vivo identification and characterization of malignant and pre-malignant tissues in the upper gastro-intestinal (GI) tract. While presence of Barrett's mucosa is simple to detect endoscopically, at the present time dysplasia and early cancer is found only by extensive biopsies. The typical protocol is four quadrant biopsies at 2-cm intervals of the Barrett's mucosa. While this is the standard technique, it only provides 3-5 % sampling of the mucosal surface where dysplasia and diffuse cancer may be found. The remaining 97-95% of the mucosa is not sampled.

Laser-induced fluorescence (LIF) spectroscopy has already been used to detect cancer and high-grade dysplasia in Barrett's esophagus. However, that system uses a contact technique, which samples a 1-mm area of tissue at each measurement. While the contact LIF system is better than the pinch biopsy technique, a new system is needed to allow examination of the entire surface of the mucosa. To address this important need in imaging, we will develop a real time synchronous imaging system based on state-of-the-art tunable filters coupled to an endoscope.

A unique MSI technology using the SL technique will be developed to obtain spatially resolved images of the slight differences in luminescent properties of malignant versus non-malignant tumors. This will provide a faster and more accurate in-vivo analysis without biopsy. The unique imaging aspect of this MSI system will provide real-time spatial information, allowing for comprehensive diagnosis of large areas of interest.

An interdisciplinary approach will be used to perform the proposed research to provide results in an efficient and cost effective manner. We will be working in close collaboration with the University of Tennessee (UT) School of Veterinary Medicine, and medical researchers with expertise in clinical studies at the Thompson Cancer Survival Center (TCSC). Following development of this technology, initial studies will be performed on two model systems, biopsied tissues as well as laboratory animals at Oak Ridge National Laboratory and UT. Once the system has been optimized, clinical in-vivo studies will be performed on human subjects at the TCSC in Knoxville, Tennessee.

STATUS OF RESEARCH AND PARTNERSHIP

During this reporting period, we have made significant advancements in several aspects of the project. We have completed the development of an in-vivo SL-based system. This system employs an optical parametric oscillator (OPO) laser as the excitation source, an emission fiber bundle for signal collection, an endoscope for delivery of the system into the esophageal tract, a liquid crystal tunable filter

(LCTF) for wavelength selection, and an intensified charge-coupled device (ICCD) for fluorescence detection.

We have successfully developed, evaluated, and tested the SL system using mice at ORNL. To simulate cancerous conditions inside the esophageal tract, nude mice were injected subcutaneously with 100 µL of Fischer 344 rat tracheal carcinoma cells (IC-12) to induce tumor formation. The mice were nude to prevent hair from interfering with our measurements and were immuno-compromised to aid with tumor formation. The subdermal injection was performed as close to the skin surface as possible to allow tumor formation close to the skin surface as is the case with an esophageal cancer. This also allowed experiments to be performed in-vivo rather than after tissue extraction. After injection, the nude mice were incubated for a period of four days to allow tumor formation to occur. Once a tumor was observed visibly (approximately 5-mm diameter), the mice were anesthetized to permit data collection.

To assist the system with image acquisition and analysis two image analysis programs have been developed. The first program collects images from the ICCD and CCD cameras and saves them into a designated file structure. The second program performs analyses on the saved images. The analysis program performs a ratiometric analysis as well as a difference analysis. These analyses provide a predictive, quantitative, diagnostic result for the data being analyzed. A malignancy trends the ratiometric analysis toward zero while trending the difference analysis towards negative infinity. During human clinical trials, we will further investigate this important feature and it is hoped that a relationship between the ratiometric analysis and cancer progression can be established and that the degree of negativity of the difference graph will correlate to cancer progression.

We are working closely with our partners and co-PIs, Drs, M. Panjehpour and B.F. Overholt at the Thomson Cancer Survival Center (TCSC), and Dr. R. DeNovo at the University of Tennessee-Knoxville (UTK) in this project. We have performed canine trials at the College of Veterinary Medicine at UTK. Various known biomarkers such as porphyrin, riboflavin and FAD present in the canine esophageal tissues were clearly detected. The fluorescence profiles of normal tissue in the canine GI tract exhibited spectral profiles similar to those we have recorded with normal tissues from human esophageal measurements. These trials have proven the efficacy and safety of the endoscopic system for future clinical use.

We have performed initial testing on humans having esophageal cancer using our SL point spectroscopic system and the MSI device. The early results of this experimentation look promising with a high degree of correlation evident between the human data and results obtained in previous works. The maximum peaks in the data from each wavelength (400 nm, 420 nm, 440 nm, 460 nm, and 480 nm) were combined to display the data. The results shown are for data processed using the differential normalized fluorescence (DNF) technique. Notice that normal squamous epithelial cells in the esophagus are clearly differentiated from Barrett's esophagus and that Barrett's esophagus is clearly differentiated from any type of dysplasia. The preliminary results of our clinical studies are very encouraging and indicate that, as the malignancy progresses through subsequent stages, the graph trends toward negative values. These data demonstrate that the SL technique has the potential to diagnose early cancer such as esophageal dysplasia. We have also performed clinical studies and conducted similar human clinical trial as described above during using the MSI imaging instrument.

ISSUES
Not applicable.

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PROJECT TITLE: Wavelength Quantum Dot-Based Probes for Cell Tracking

PARTNERS' NAMES AND AFFILIATIONS:
Quantum Dot Corporation

GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering

ABSTRACT

Purpose: We extended our work using quantum dots provided by Quantum Dot Corporation (QDC) to investigate their use in tissue engineering and labeling in tissue-cultured cells and in vivo.

Methods and results: The newer generations are brighter (quantum yields in excess of 90% are common), more stable, and more uniform in size. The amphiphilic primary coat developed by QDC has proved to be adaptable for many uses (see below). Most important, we have begun use of the next generation of near-infrared emitting quantum dots (of which the first were 705 and 755nm emitting). These new Qdots have made in vivo visualization much easier.

We have used quantum dots having a primary coat of an amphipathic polymer and several secondary coats to evaluate the effect of different secondary coats and surface charges on the distribution of quantum dots injected intraveneously and in the interstitial space of various tissues.

On intraveneous injection, quantum dots are taken up by the RES; relative retention of quantum dots in different RES organs is influenced by the surface coat. Coating quantum dots with high molecular weight PEG coats results in long serum lifetimes, and minimizes trapping in lymph nodes. Either carboxyl- or amino-surfaces cause higher uptake by the lymph nodes and much reduced serum lifetimes, even when the charged groups are at the distal ends of attached PEG. Organs clear in the order liver>bone marrow>spleen>lymph nodes. After several months, essentially all fluorescence is confined to the lymph nodes. Most quantum dots are excreted in the feces.

We injected quantum dots into mouse interstitium at various sites, then followed uptake and retention into the lymphatic system. Some quantum dots always remain at the site of injection; but uptake into adjacent and further lymph nodes is rapid, and may be followed non-invasively.

Direct injection into tumors using quantum dots having three different charged surfaces showed that all migrated to adjacent (sentinel) lymph nodes within a few minutes. Uptake into lymphatic vessels and transfer to successive nodes may be monitored easily and non-invasively. Fluorescent quantum dots are retained by lymph nodes for at least two years, thus potentially very long-term monitoring of labeled cells is possible.

We have also used quantum dots to explore cellular uptake and cell tracking. We extended our initial work on cell labeling using (1) PEG-Qdots for labeling dendritic cells and macrophages (2) cationic lipid-coated Qdots for fibroblasts, and (3) streptavidin-coated Qdots pre-reacted with a biotinylated poly-arginine peptide for fibroblasts, osteoblasts and epithelial cells. In the latter case, cells were labeled with up to four different colors (green (525 nm emission; yellow(592 nm emission); orange (611 nm emission) and red (655 nm emission) of Qdots. All three methods worked for their respective cell types (Lagerholm et al., Nano Letters 4, 2019). In all cases, cellular uptake appears to be via endocytosis. Not only are the Qdots very bright, but fluorescence persists for several generations after cellular uptake. These methods have now been commercialized by Quantum Dot Corporation.

We mastered injection of Qdots into the vasculature system of developing chick embryos. This is part of a collaborative project with tissue engineers at Carnegie Mellon who are determining how the vascularization of engineered tissue depends on polymer scaffold and growth factor composition. Engineered matrices are implanted onto the chick chorioallantoic membrane. Effect of gradients of growth factors and vascularaization can be followed with much greater ease and clarity than hitherto possible (submitted for publication).

Conclusion: We find that infrared quantum dots with appropriate coatings are very useful for in vivo imaging. However, the utility depends strongly upon selecting or developing the appropriate coating.

STATUS OF RESEARCH AND PARTNERSHIP

The partnership has worked well. Weekly phone calls and twice-yearly visits maintain good communication. The project is on schedule.

ISSUES

None at the present time.

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PROJECT TITLE: Cardiopulmonary Organ Engineering

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT

The aim of this proposal is to design solutions for vascular, cardiac, and pulmonary organ failure by building interactive teams of researchers focused on specific aspects of cardiopulmonary organ engineering. Our efforts will encompass three projects: a tissue engineered blood vessel, a myocardial patch, and a biohybrid lung. The assembled research teams will function as cores of expertise that address common tasks associated with all three projects. Five research cores will be established in the following areas: 1) matrix synthesis and surface modification, 2) precursor cell isolation and characterization, 3) biomechanical testing and conditioning, 4) animal model development, and 5) construct assessment. For each of the three organ projects we have design objectives (Specific Aims) that will be achieved in the five-year period of proposed work: 1) Tissue engineered blood vessel - A biological blood vessel will be developed that achieves long-term potency in the rat model and is subsequently evaluated in the porcine model. The blood vessel will be a "biological equivalent" to autologous arteries from a mechanical and biofunctional perspective. During vessel development in vitro, specific mechanical training protocols that have been optimized to direct appropriate cell differentiation and expression of matrix components will be employed. 2) Myocardial patch - A process will be developed that allows the reconstruction of functional myocardium in ischemic or dysfunctional regions of the heart, This process will be characterized by the seeding of stem cells onto a bioerodible thermoplastic elastomer which has been designed to micromechanically transmit appropriate stresses to the stem cells during an in vitro seeding period and after placement within the diseased myocardium. Vascularization of this implanted construct will be achieved by surgical placement of omental tissue atop the placed myocardial patch. 3) Biohybrid lung - An oxygenator comprised of endothelialized microporous hollow fibers arranged in: plates and rotated to mix and pump the blood will serve as a biohyrid lung capable of providing gas exchange in a calf for 14 days. The hollow fibers will be surface modified to support the culture of autologous endothelial cells. The endothelial cells will act to reduce the anticoagulation requirements of the device while maintaining adequate fiber permeability.

STATUS OF RESEARCH AND PARTNERSHIP

For the myocardial patch our work has focused on developing our rat surgical model and then proceeding to investigate our best scaffolds to date (developed by the Matrix Synthesis Core) as a full thickness replacement of the rat right ventricular outflow tract. Briefly, we utilized a biodegradable poly(ester urethane)urea (PEUU) that was processed by thermally induced phase separation into an open pore scaffold, which was used as a full wall thickness replacement of the outflow tract in Lewis rats. For control purposes we used the clinically relevant expanded poly(tetrafluoroethylene) (ePTFE) porous

material. At 3 months the biodegradable scaffold had degraded nearly completely in vivo with a mild inflammatory response and showed excellent surgical handling properties. Subsequently we have moved to using the patch as a partial-wall replacement over left ventricular infarcts in the rat model and shown improved cardiac function with the scaffold patch. In work with muscle-derived stem cells (MDSCs), the Huard lab has performed more extensive related work with mouse MDSCs injected into the heart and has begun experiments with rat MDSCs injected into healthy myocardium. Results from this work show some evidence of MDSC differentiation to or fusion with cardiomyocytes, further experimentation is ongoing.

Related to the construction of a tissue engineered vascular graft (TEVG) we have started utilizing a tubular biodegradable PEUU polymer fabricated by the Matrix Synthesis Core and seeded with mouse muscle-derived stem cells (mMDSCs) provided by the Precursor Cell Isolation Core. The constructs have an inner diameter of 3.5 mm and a wall thickness of 200-300μm. In order to incorporate the cells into the scaffolds, we have developed a new device that is currently under provisional patent review. The seeding device utilizes simultaneous vacuum and rotation to provide a uniform, reproducible "bulk" seeding of a porous tubular scaffold; i.e., even distribution in both radial and longitudinal dimensions. Importantly, the seeding procedure is completed in 1 to 2 minutes. The device is currently under optimization using a validated computational fluid dynamic model. Seeded PEUU constructs have been dynamically cultured in a spinner flask for 3 and 7 days. Following 3-day culture, the cells proliferated and were evenly spread through the wall thickness. When cultured in ascorbic acid supplemented media, we observed increased proliferation and collagen production after 7 days. We also have begun exploring the utility of the device as a means for fast and efficient luminal surface seeding.

In work focused on further characterization of precursor cells, research into the utility of surface markers is ongoing. We have investigated in vitro cell behavioral characteristics under imposed conditions that challenge the propensity of myogenic progenitors to choose among various cell fates (e.g., proliferation, quiescence, and differentiation). Previous observations in mice have suggested an enhanced in vivo regenerative capacity of myogenic populations with respect to their in vitro ability to maintain a proliferative and undifferentiated state. Based on these observations, our hypothesis is that such behavior may constitute an a priori indicator of regenerative capacity after transplantation. To test this proposition, we evaluated a rat cell isolation and transplantation model via the same protocol used for mice. The rat model results paralleled those observed in the mouse model, revealing a significant correlation between regenerative capacity and the induction of differentiation.

In our matrix synthesis activities, we have synthesized a growing family of biodegradable poly(urethane)ureas that act as thermoplastic, biodegradable elastomers. These polymers have been characterized chemically and mechanically and have been processed using a variety of techniques. The mechanical properties have proven to be generally very attractive with high tensile strengths and high distensibilities. Scaffolds have been formed from blends of the polymers with collagen and growth factors and shown to exhibit enzymatic-sensitive degradation and bioactive growth factor release over a three-week period. Our scaffold development efforts have been closely linked to our biomechanical testing and conditioning core. In this core, bioreactors have been developed to provide ongoing loading of cell-seeded scaffolds. Techniques have also been developed for the measurement of scaffold micromechanical properties and to study relationships between scaffold structural anisotropy and mechanical behavior.

ISSUES

Our activities have been productive and we have established effective collaborative mechanisms and an effective administrative reporting structure.

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PROJECT TITLE: Biomimetic Blades: Mincing With Less Mineral

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GRANTING NIH INSTITUTE/CENTER: National Institute of Dental and Craniofacial Research

ABSTRACT
Tooth enamel and dentin are the premier materials made by vertebrates for hardness and abrasion resistance. The superb properties of these materials are vital adaptations for proper ingestion nutrition and, when compromised through decay or injury, pose many fundamental and technical challenges to effective restoration. In polychaete worms such as Glycera and Nereis, the tooth-like jaws have a resistance to wear that is comparable to enamel; however, this is accomplished with a tenth as much mineralization (Glycera) or no mineralization at all (Nereis). These mainly proteinaceous jaws offer important insights into the design of biocompatible wear-resistant materials. Based on preliminary studies, we propose to demonstrate that specific proteins/polymers can be hardened and toughened by slight mineralization, metal ion chelation, or both. Our aim in this discovery-driven proposal is a state-of-the-art chemical, structural and mechanical characterization of the jaws using mass spectrometry, molecular biology, X-ray analysis and nanoindentation. Rigorous engineering principles will be applied to the analysis of jaws to distill a set of biomimetic rules regarding the relationship between structure and wear. Significant correlations between the chemical, microstructural and mechanical properties will be used to direct the preparation of His-containing copolymers into hard films containing Cu or Zn ions. The chief health benefits of this research will be insights about lightweight replacement materials with superior hardness and abrasion resistance.

STATUS OF RESEARCH AND PARTNERSHIP
In the past twelve months research has progressed significantly on three fronts: molecular, chemical and mechanical. Using PCR with molecular primers based on peptide sequences obtained from protein extracted from Nereis jaws, two unusual precursors have been characterized: a 35 kDa protein with nearly 27 mole% histidine and 36 % glycine; as well as a smaller tyrosine rich protein at 15 kDa. Together the proteins resemble the composition of the jaws. The His-rich Nereis protein consists of numerous HHGGH repeats that will be assessed for their ability to bind Zn. Nereis jaws also contain significant levels of bromine and iodine; most is covalently bound to the His and Tyr residues of proteins. In Glycera jaws, 35-40 weight % of the jaws was found to be eumelanin.

This period has also seen completion of the construction and testing of a wet cell for use in nanoindentation. Hydrated jaws of both Nereis and Glycera show a hardness and stiffness that is 10-30 % lower than the dry specimens with the tips showing the least change. Removal of all protein by extended hydrolysis allowed testing of the substantial melanin rich residue in Glycera jaws. This retains close to 100% and 60%, respectively, of the original stiffness and hardness of the intact jaws, and is the first known melanin to exhibit a load-bearing capacity. Removal of zinc from Nereis jaws by EDTA chelation results in a loss of 90 % hardness in the core regions, but the margins (surface to a depth of 4 microns) are less affected perhaps, as EDS maps suggest, because the zinc is significantly more difficult to remove. We propose that halogenated ligands in the margin may provide stronger binding to zinc.

Careful scrutiny of the Glycera and Nereis jaws reveals that both contain a pellicle that is amorphous in contrast to the fibrous anisotropic interior.

ISSUES

Research is not progressing with equal speed in all areas. Microanalysis of organic components in macromolecular structures is definitely lagging. A specific example is the need to detect histidine and tyrosine concentrations with microscale resolution. We are exploring partnerships with labs that are pioneering characterization of ligand chemistry with high spatial resolution (synchrotron EXAFS). Also given the extensive knowledge we now have about sequence, chemistry and mechanics, it is time to find partners for synthesis of His-rich and halogenated metal binding proteins. This aim was scratched from the original proposal because it was felt we had an insufficient database to pursue synthesis. This is no longer the case.

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PROJECT TITLE: High Resolution SPECT/CT Imaging of Systemic AA-Amyloidosis in Mice

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GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke and National Institute of Biomedical Imaging and Bioengineering

ABSTRACT

High resolution imaging is becoming an invaluable tool in biomedical research much as it has to the clinician. In the clinic, imaging offers a precise, non-invasive means of diagnosis and directly influences both the therapeutic approach and prognosis. Unfortunately, the development of high-resolution imaging tools demanded by researchers has lagged behind that of the clinic; thus, characterization of the kinetics of in vivo pathology and the subsequent development of novel, effective therapeutics has been hampered. This is particularly true in the field of amyloid-related diseases which include Alzheimer's disease, type II diabetes and primary (AL) amyloidosis. It is impossible to fully appreciate and understand the complexity of these diseases, and the means by which they may be halted, without the ability to perform longitudinal studies in individual animals in vivo. To that end, the development high-resolution micro-imaging technologies capable of detecting and quantifying amyloid deposits in vivo is warranted and imperative. We intend to address these important issues through the design and application of a powerful new dual-modality imaging technology, microSPECT, combined with microCT, supported by state-of-the-art 3-D image reconstruction and analysis software. This new technology will be employed to identify radiolabeled amyloid deposits in live animals and present the amyloid distribution within the context of a high-resolution CT image of the visceral terrain. With this technology, the goal of quantifying organ-specific amyloid burden in vivo is attainable. The goals are thus to: (i) Complete the design and implementation of a high-resolution, small-animal specific dual SPECT/CT imaging system. (ii). Develop a system of amyloid quantification in which microSPECT image data can be directly correlated to amyloid burden. (iii) Use these technologies to study the progression of systemic AA-amyloidosis in two murine models and the regression thereof in response to novel immunotherapies. This study will not only result in technological advancements in the field of small-animal imaging and amyloid-specific radio-tracers but will also provide a wealth of information on the natural progression of amyloidosis in vivo and establish a paradigm for the screening of therapeutic drugs in animal models of human disease. Furthermore, the translation of amyloid-specific imaging technologies will yield tangible clinical benefit.

STATUS OF RESEARCH AND PARTNERSHIP

Biology and Radiochemistry: At the end of yr. 2 SPECT detectors had been fabricated and were mounted on an independent image acquisition system that was housed immediately adjacent to the microCT apparatus in ORNL. By the end of yr. 3 a dual-head microSPECT/CT hybrid machine had been fully assembled, tested, and is now commercially available through Siemens Medical Solutions Molecular Imaging (Knoxville, TN). In the last 12 months, we have evaluated two new tracers for amyloid in addition to our continued use of ^{125}I-labeled serum amyloid P component (SAP). The first, a monoclonal

antibody generated in our laboratory using marmoset AA-amyloid deposits as an immunogen binds AA amyloid fibrils in the marmoset, cow and human. In addition we have tested ^{125}I-labeled bovine aprotinin (Trasylol™, Baxter Pharmaceuticals) in the mice and found that, using the protocol described for labeling amyloid in humans, only renal uptake was observed – this protocol is being re-evaluated. Using ^{125}I-labeled SAP as a tracer in mice with severe systemic AA-amyloidosis we have confirmed the presence of amyloid-bound tracer in target organs and correlated the SPECT images with biodistribution analyses, micro-autoradiography and histology-based quantitation of amyloid and found a good concordance (Amyloid: J. of Protein Fold. Disorders, In Press). With respect to our aim of performing quantitative micro-imaging of amyloid in mice we have developed a collaboration with Drs. David Townsend and Jeffrey Yap and performed a number of microPET/CT imaging experiments using the systemic AA-amyloid mouse model and the positron emitting isotope ^{124}I conjugated using identical chemistry to SAP. These studies have provided high resolution images of amyloid in the mice and we have used this model to develop a simple method for determining whole body clearance in individual mice. We have performed a number of post mortem studies with ^{124}I-SAP and generated hi-resolution co-registered PET/CT images that unequivocally identify amyloid deposits in the liver, spleen and, for the first time, the heart. In recent months we have performed dual-modality (PET/CT) live animal imaging to monitor the uptake and clearance of ^{124}I-SAP in organs with and without amyloid deposits. The image data have been validated using whole body activity determinations. We will continue to use microPET imaging in addition to SPECT to provide quantitative organ-specific localization of amyloid in the mice. Although PET is considered a more sensitive and quantitative modality than SPECT there are certain advantages of using ^{125}I, namely its long half life that permits micro-autoradiographic analyses which are used as definitive validation for the tracer distribution in the images therefore this isotope will also remain in use in our studies.

   MicroSPECT Systems Development: The dual SPECT/CT apparatus is now commercially available through Siemens Medical Solutions Molecular Imaging (Knoxville, TN)

ISSUES

   Several key members of our research team went on entrepreneurial leave from ORNL in order to produce and market the microCT and dual microSPECT/CT hybrid machines. This transition led to the involvement of new members of the BRP but continued successes.

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PROJECT TITLE: Functional Brain Imaging by Laser-Induced PAT

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GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke

ABSTRACT
High-resolution optical imaging beyond 1-mm imaging depth is a void. The objective of the proposed research is to develop a novel non-invasive laser-based technology for transcranial functional imaging of the brain of small animals in vivo. Small animals are the preferred laboratory models for studying various diseases, and small animal imaging provides the opportunity to evaluate pathologic progression in a much-compressed time frame and with a much-improved resolution. By combining high optical contrast and high acoustic resolution, the proposed technology, functional photoacoustic tomography (fPAT), can image the intact brain free of speckle artifacts. Besides structural information, the proposed fPAT can also provide functional information including total hemoglobin concentration and blood oxygenation.

In the proposed fPAT technology, a short-pulsed laser beam penetrates into the tissue sample diffusively. The photoacoustic waves, due to thermoelastic expansion resulting from a transient temperature rise on the order of 10 mK caused by the laser irradiation, are then measured around the sample by wide-band ultrasonic transducers. The acquired photoacoustic waves are used to reconstruct, at ultrasonic resolution, the optical absorption distribution that reveals optical contrast.

Optical contrast is sensitive to the molecular conformation of biological tissue and is related to certain physiological parameters such as the level of hemoglobin oxygenation. The proposed fPAT technology combines the high-contrast advantage of optical imaging with the high 3D resolution advantage of ultrasound imaging. The proposed technology does not depend on ballistic/quasi-ballistic or backscattered light as optical coherence tomography (OCT) does. Any light, including both singly and multiply scattered photons, contributes to the imaging signal; as a result, the imaging depth in fPAT is better than in OCT. The resolution is bandwidth-limited by the detected photoacoustic waves rather than by optical diffusion; consequently, the resolution of fPAT is excellent and scalable with the desired imaging depth. Furthermore, fPAT is free of the speckle artifacts present in OCT and pulse-echo ultrasonography, two analogous technologies. In conclusion, fPAT fills the void of high-resolution imaging beyond the 1-mm imaging depth.

STATUS OF RESEARCH AND PARTNERSHIP
So far, we have performed functional photoacoustic imaging of small animal brains in vivo to demonstrate the capability of PAT to assess simultaneously cerebral blood volume and oxygenation. Transcranial imaging of two functional parameters, the total concentration of hemoglobin and the oxygen saturation of hemoglobin, in small-animal brains was realized in vivo non-invasively by laser-based photoacoustic tomography for the first time to our knowledge. Multi-wavelength spectroscopic photoacoustic tomography can be used to assess the optical absorptions of endogenous chromophores,

e.g., oxygenated and deoxygenated hemoglobins, while its spatial resolution is bandwidth-limited by the photoacoustic signals rather than by optical diffusion.

We have also achieved molecular imaging based on our noninvasive in vivo spectroscopic photoacoustic tomography. We are able to image the level of the targeted contrast agent accumulation in a mouse brain tumor provides direct information related to the physiopathological processes in the tumor. Molecular spectroscopic PAT, which is based on the spectroscopic differences among oxygenated hemoglobin, deoxygenated hemoglobin and the contrast agent, can be used to image not only the contrast agent accumulation but also the total hemoglobin concentration and tumor hypoxia.

This technique, with its prominent intrinsic advantages, can potentially accelerate the progress in neuroscience and lead to a better understanding of the interrelationship between neural, hemodynamic and metabolic activities in the brain.

ISSUES

There was an error in accounting when the first-year funds reached the PI's institution. Consequently, the subcontract to the partner at University of Connecticut, who is responsible for the construction of an ultrasonic array system, was deferred. As a result of this delay, we expect a delay in the delivery of the ultrasonic array.

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PROJECT TITLE: Multifunction Prosthesis Control Using Implanted Sensors (1 R01 EB01672-01)

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering and National Institute of Child Health and Human Development

ABSTRACT

The limitation of current prostheses is not the devices themselves but rather the lack of sufficient independent control sources. A system capable of reading intra muscular EMG signals would greatly increase the number control sources available for prosthesis control. Current state-of-the-art electric prosthetic hands are generally single DOF (opening/closing) devices often implemented with EMG control. Current prosthetic arms requiring multi-DOF control most often use sequential control. As currently implemented, sequential control is slow.

We propose to develop a multichannel/multifunction prosthetic hand/arm controller system capable of receiving and processing signals from up to sixteen implanted bipolar differential electromyographic (EMG) electrodes. An external prosthesis controller will use fuzzy-logic to decipher user intent from telemetry sent over a transcutaneous magnetic link by the implanted electrodes. The same link will provide power for the implanted electrodes.

- Northwestern University will develop the multifunctional prosthesis controller and perform the animal experiments necessary to demonstrate the implanted devices.
- Rehabilitation Institute of Chicago will perform animal experiments and help with human subject experiments.
- Illinois Institute of Technology will develop individually addressable integrated circuit EMG sensor packages. Each sensor will be housed in BION® hermetically sealed packages provided by the Alfred E. Mann Foundation.
- Sigenics Corp. will develop the transcutaneous telemetry link, (or reader). A custom-designed application-specific integrated circuit (ASIC) will "strip" the data from the link's telemetry and send it to the prosthesis controller. Powering of the implanted electrodes will also be controlled by the ASIC. The external coil of the inductive link will be laminated into a prosthetic socket.

STATUS OF RESEARCH AND PARTNERSHIP:

Controller Algorithm Development. We are now 1 3/4 years into this project. In that time I believe good progress has been made. We have conducted extensive human subject experiments in an effort to elucidate the best method of control to use to integrate the contributions from the 16 different implanted sensors. The multi-channel/multifunction prosthetic hand/arm controller must be capable of receiving and processing signals from the implant telemetry system. This same controller must then decipher user intent from the telemetry sent by the IMES to decide which actuators in the prosthesis to drive.

The simplest control paradigm is to use one muscle to control one function in the prosthesis [one muscle - one function]. The implicit assumption underlying this approach is that a contracted muscle EMG signal power is much greater than the relaxed muscles EMG signal powers i.e. high SNR. We anticipate this type of control will be possible for the shoulder and the elbow. This type of control is problematic for the wrist and hand because multiple muscles control the same functions-the distal arm is an indeterminate system.

A more sophisticated approach is to recognize patterns/features of EMG activity associated with different training movements and/or functions and have the controller drive the appropriate prosthesis actuators [i.e. one pattern of EMG activity - one function]. The control is intuitive and easy to remember since users execute the movement the prosthetic limb is to perform with their "phantom limb." Recognition is achieved through feature extraction algorithms, artificial neural nets, or some other similar high level classification method. We have been recording the patterns of EMG activity associated with different training movements and/or functions and then training the controller to recognize these patterns. We have explored recognition through automated classification techniques using neural networks, linear discriminant anslysis, fuzzy clustering, and multivariate linear regression. This approach requires a pattern to be stored for every desired movement. The problem with these approaches is that they are still task-based control systems. The user is still compelled to think in terms of what grip they need to do rather than subconsciously reaching out and picking up an object. We have yet to draw any definitive conclusion as to which classification technique we prefer, but the fuzzy clustering method does lend itself to a default "safe" [fail safe] approach when considering driving motors that are inherently non-backdrivable (as are commonly used in prosthetics to preserve power and to hold commanded position and force in the absence of power).

System Architecture Development. Development of the system is well under way. We are assembling new Class-E exciter modules to test the new implants. PC boards are in hand waiting for the new Class-E Controller chip. We will continue the magnetics design to verify reception of outward telemetry. Next we need to Release the official Interface Control Document (ICD), which completely specifies the interface between the telemetry controller (MSP430) and the prosthesis controller. So that the prosthesis controller can take the data sent from the implant system and use it to control the prosthesis. To date, we have submitted three fabrication runs on the XFab CX08 process. Devices from the first run were tested with encouraging results. We have chips back from the second run and these are currently in test. These chips are in their near final form factor to fit in the AEMF capsules, however, initial tests have revealed some non-insurmountable defects that are being corrected in a number of up coming wafer runs. The near future goal for the hardware development is an end-to-end demonstration of the system.

In other work we simulated the pickup area for our implants, leveraging off work being done at Dr. Kuiken's lab on electromagnetic finite element modeling techniques. The purpose of this study was to model the feasibility of recording independent EMG signals from the muscles of the forearm, for myoelectric prosthetic control, using chronically implanted IMES electrodes. The simulation results suggest that the presence of a thin layer of encapsulation tissue around the IMES should not impede the detection of EMG signals from the surrounding muscle fibers and may in fact cause the amplitude of the EMG signal to increase modestly. The orientation of the bipolar electrode with respect to the fiber direction is an important factor in determining the selectivity of the implanted electrode. Alignment of the electrode along the fiber direction will be particularly important in smaller muscles. We found that for an implant placed along the fibers of the muscle in which it is inserted the pickup area for the sensor will be a cylinder about 5mm in radius about the implant.

ISSUES

We got a false start with the human subject experiments due to equipment issues with the intramuscular EMG measurement system. These have been solved.

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PROJECT TITLE: Ophthalmic Imaging Using Adaptive Optics and OCT

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GRANTING NIH INSTITUTE/CENTER: National Eye Institute

ABSTRACT
 The purpose of this BRP is to develop and evaluate new optical instrumentation that will permit unprecedented three-dimensional, in vivo, imaging of single cells in the human retina, specifically rod and cone photoreceptors and ganglion cells. An interdisciplinary team is combining adaptive optics (AO), enabling the best lateral resolution for retinal imaging, with optical coherence tomography (OCT), providing the best axial resolution for retinal imaging. This instrumentation will be used to study cellular morphology associated with normal aging, age-related macular degeneration and glaucoma.

STATUS OF RESEARCH AND PARTNERSHIP
 This BRP is nearing the completion of the second year of funding. The AO arm for the core design of an AO-OCT system was completed first in a flood-illuminated retinal imaging system, and then with AO-OCT. Both types of system have been constructed at the UC Davis and Indiana University sites in collaboration with engineers from the Lawrence Livermore National Laboratory.

 During this year, the BRP has extensively evaluated wavefront correctors for the AO arm, particularly a bimorph deformable mirror (DM) because of its lower cost and high dynamic range than conventional DMs. These tests demonstrate that this mirror has considerable promise in human retinal imaging with AO. While some tests in a closed-loop correction are continuing, interferometric characterization has been published and tests have been carried out in the UC Davis AO-OCT system.

 A large number of improvements were made in the OCT systems at both UC Davis and Indiana University. Using Fourier-domain OCT (also referred to as spectral-domain or SD), it was possible to achieve axial resolution of 3.5 μm and, in collaboration with retinal specialists, to identify retinal structures not previously noted in the OCT literature. High speed (50 μsec exposures) permitted acquisition of hundreds of B-scans for rendering 3D volumes using custom software developed with computer scientists at UC Davis. Further work will be carried out in the next project period with 3D rendering to explore potential clinical applications of AO-OCT.

 Both the UC Davis and the Indiana University sites have now obtained AO-OCT images to take advantage of the high lateral resolution made possible by AO and the high axial resolution and sensitivity made possible by OCT. Each BRP site has, however, used a different OCT instrument design.

 In year two Indiana University completed development and evaluation of a novel AO parallel SD-OCT retina camera that aimed to achieve the necessary optical resolution, sensitivity, and imaging speed to observe single cells in the living human retina. Axial resolution and sensitivity were realized with SD-OCT; lateral resolution was provided by AO. High imaging speeds were accomplished with a free-space parallel illumination architecture, an approach that complemented the scanning design pursued by UC Davis. The flood-illumination approach carried noticeable risk as it had not been previously applied to the retina or combined with AO. Indiana University therefore chose also to develop in year two a less risky scanning SD-OCT camera (without AO). The scanning system could be interchanged with the

flood illumination system in the event the flood system failed to meet the imaging requirements for the proposed BRP vision science experiments.

Rigorous evaluation of the AO parallel SD-OCT instrument in year two included: quantifying its 3-D optical resolution (3.0 x 3.0 x 5.7 µm in the living human eye); measuring its sensitivity (up to 94 dB); optimizing image acquisition speed (up to 150,000 A-scans/sec); quantifying impact of ocular aberrations and induced defocus on camera sensitivity (more than 10 dB change); comparing retinal images to that acquired with commercial and established research-grade OCT instruments; and evaluating retinal images for cellular structures (e.g., observing the interface between the inner and outer segments of individual photoreceptor cells resolved in both lateral and axial dimensions). A published paper demonstrated the improvement in image resolution with AO parallel SD-OCT (with and without AO) compared to commercial, and research-grade scanning OCT cameras.

ISSUES

Three issues are: (1) Speckle noise results from the coherent nature of the OCT detection method and results in a reduction of image quality. Several approaches for reducing speckle noise are being evaluated. (2) 3D reconstructions will permit new insights for basic research and will be especially important for evaluation of combined AO-OCT imaging. Further improvements in image segmentation of the 3D volumes will be critical for clinical application. (3) The natural movements of the eye, even with visual fixation, limit the resolution obtainable with any imaging system. For this reason, future efforts will attempt to further improve image acquisition speed.

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PROJECT TITLE: Methods in Molecular Imaging and Targeted Therapeutics

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT
   The broad subject of this Biomedical Research Partnership (BRP) application is the development of novel multidimensional nanotechnologies for sensitive and specific imaging of molecular epitopes that are etiologic for atherosclerosis. The unifying hypothesis is that targeted molecular imaging with novel contrast agents can delineate selected molecular features of atherosclerotic lesions that are critical determinants of early lesion growth and later lesion instability. Noninvasive and early detection of these situations could enhance patient management and potentially reduce the incidence of myocardial infarction and stroke. The long-range goal is to produce a targeted nanoparticle contrast agent characterized by: 1) flexible targeting options depending on the binding ligand selected, 2) flexible imaging choices based on the contrast mechanism best suited to the pathology in question, and 3) flexible opportunities for local delivery of therapeutic agents coupled directly with image-based quantification of local nanoparticle deposition. The technology is expected to enable early noninvasive detection of a variety of pathologies, convenient serial outpatient evaluation, and site-targeted delivery of therapeutics as clinically indicated. Stable and safe self-assembling nanoparticles will be developed, refined, and tested for visualization of pathological epitopes with the use of magnetic resonance imaging (MRI).

STATUS OF RESEARCH AND PARTNERSHIP
   Development of unique molecular targeting ligands for characterizing early and unstable atherosclerosis: Although little of this aim is the subject of academic publication due to its technical and developmental nature, a substantial progress has been achieved in conjunction with our partners at Kereos, Inc. and Dow Chemical resulting in several patent applications. Several key issues that critically impact the development of the targeted MRI agent have been addressed and are either resolved or completed. Critical to the effort was the development of a gadolinium-based agent (Gd-API) that meets the needs of product stability and efficacy, Gd-API stability (no Gd dissociation), assessment of legal freedom to operate, synthesis method (ready to initiate GLP toxicology lot synthesis), and analytical Gd release test methods. We now have in hand two agents (a proprietary MeO-DOTA agent, and a congener) that meet these criteria. We have developed their synthesis and analytical chemistry to the point that GLP toxicology lot syntheses can be initiated. We also have developed the formulation with a cleavable linker and are in the process of evaluation of that compound.
   The second API in our 3 –targeting ligand. We have developed a very $\alpha_v\beta_3$ emulsion drug product is the much improved (as compared with the original $\alpha_v\beta_3$ mimetic we jointly developed initially with Bristol-Myers Squibb) and economical synthesis route to this rather complicated ligand. In addition, we have made minor linker modifications that simplify the chemistry and the analytical control of the product. These variations are currently being evaluated in $\alpha_v\beta_3$–binding assays so that we can choose the final structure for development; i.e., the simplest structure will be chosen if $\alpha_v\beta_3$ bindings are similar, as we anticipate. The key synthesis steps are all worked out and initial analytical for the entire process and individual steps is worked out as well. We are ready to initiate GLP toxicology lot syntheses for this API. The GLP toxicology lot synthesis will be carried out using two separate contract research organizations.
   The drug product (emulsion of consisting of the two above mentioned APIs, perfluorooctyl bromide, Egg Lecithin, glycerol and buffered water) has progressed to the stage of component optimization. Emulsions are produced using a two stage mixing protocol, homogenization using an Ultra Turrax Homogenizer followed by high shear microfluidization. Emulsions are characterized by particle size analysis, pH and composition and monitored for storage stability. Analytical techniques have been established for these analyses.

Proton-based MR approaches for 3-integrin $\alpha_v\beta_3$-detecting plaque angiogenesis in cholesterol-fed rabbits (targeting): Angiogenesis is a critical feature of plaque development in atherosclerosis and might play a key role in both the initiation and later rupture of plaques that lead to myocardial infarction and stroke. The precursory molecular or cellular events that initiate plaque growth and that ultimately contribute to plaque instability, however, cannot be detected directly with any current diagnostic modality. Atherosclerosis was induced in New Zealand White-integrin–targeted, paramagnetic $\alpha_v\beta_3$ rabbits fed 1% cholesterol for 80 days. Nanoparticles were injected intravenously and provided specific detection of the neovasculature within 2 hours by routine magnetic resonance imaging (MRI) at a clinically relevant field strength (1.5T). Increased angiogenesis was detected as a 47% enhancement in MRI signal averaged throughout the abdominal aortic wall -integrin, paramagnetic nanoparticles. $\alpha_v\beta_3$ among rabbits that received -integrin, nonparamagnetic $\alpha_v\beta_3$ Pretreatment of atherosclerotic rabbits with nanoparticles competitively blocked specific contrast enhancement of the -integrin targeted paramagnetic agent. MRI revealed a pattern of increased $\alpha_v\beta_3$-integrin distribution within the atherosclerotic wall that was spatially $\alpha_v\beta_3$ heterogeneous along both transverse and longitudinal planes of the abdominal aorta. Histology and immunohistochemistry confirmed marked proliferation of angiogenic vessels within the aortic adventitia, coincident with prominent, neointimal proliferation among cholesterol-fed, atherosclerotic rabbits in comparison with sparse incidence of neovasculature in the control animals. This molecular imaging approach might provide a method for defining the burden and evolution of atherosclerosis in susceptible individuals as well as responsiveness of individual patients to antiatherosclerotic therapies.

Proton imaging of atherosclerotic plaque: Before molecular imaging with MRI can be applied clinically, certain problems, such as the potential sparseness of molecular epitopes on targeted cell surfaces, and the relative weakness of conventional targeted MR contrast agents, must be overcome. Accordingly, the conditions for diagnostic conspicuity that apply to any paramagnetic MRI contrast agent with known intrinsic relaxivity were examined in this study. A highly potent paramagnetic liquid perfluorocarbon nanoparticle contrast agent (250 nm diameter, >90000 3+Gd/particle) was imaged at 1.5 T and used to successfully predict a range of sparse concentrations in experimental phantoms with the use of standard MR signal models. Additionally, we cultured and targeted the smooth muscle cell (SMC) monolayers that express "tissue factor," a glycoprotein of crucial significance to hemostasis and response to vascular injury, by conjugating an anti-tissue factor antibody fragment to the nanoparticles to effect specific binding. Quantification of the signal from cell monolayers imaged at 1.5 T demonstrated, as predicted via modeling, that only picomolar concentrations of paramagnetic perfluorocarbon nanoparticles were required for the detection and quantification of tissue factor at clinical field strengths. Thus, for targeted paramagnetic agents carrying high payloads of gadolinium, it is possible to quantify molecular epitopes present in picomolar concentrations in single cells with routine MRI.

Fluorine imaging of atherosclerotic plaque: Previous work has shown that fibrin-targeted, liquid perfluorocarbon nanoparticles, which carry a high payload of gadolinium, have a high sensitivity and specificity for detecting fibrin with clinical 1H MRI. In this work, the perfluorocarbon content of the targeted nanoparticles is exploited for the purposes of 19F imaging and spectroscopy to demonstrate a method for quantifiable molecular imaging of fibrin in vitro at 4.7 T. Additionally, the quantity (concentration, in picomoles) of bound nanoparticles formulated with different perfluorocarbon species was calculated using 19F spectroscopy. Results from this manuscript indicate that the high degree of nanoparticle binding to fibrin clots and the lack of background 19F signal allow accurate quantification using spectroscopy at 4.7 T, as corroborated with proton relaxation rate measurements at 1.5 T and trace element (gadolinium) analysis. Finally, the extension of these techniques to a clinically relevant application, the evaluation of the fibrin burden within an ex vivo human carotid endarterectomy sample, demonstrates the potential use of these particles for uniquely identifying unstable atherosclerotic lesions in vivo.

ISSUES

The signficance of the progress in this BRP is that a final formulation is on hand that will serve as a GMP source for clinical trials of a paramagnetic angiogenesis imaging agent with multispectral imaging capabilities. Furthermore, we are pursuing pathways for multiple ligand conjugations (mimetics, mAb) and multiply targeted particles with the potential for local drug therapy as well as rational drug dosing based on imaging readouts of bound nanoparticulate agents. The new phamacokinetics/pharmacodynamics for targeted nanoparticle agents will enhance the ability to tailor diagnosis and therapy to individual patients (i.e. true "personalized" medicine).

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PROJECT TITLE: Adaptive Optics Instrumentation for Advanced Ophthalmic Imaging

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Eye Institute

ABSTRACT
The goal of the BRP is to marry adaptive optics and confocal imaging to create initially 4 instruments for noninvasive imaging of the retina with unprecedented spatial resolution. The four instruments are:

1. An AOSLO, capable of fluorescence imaging, that can image eyes, such as those of rat, dog, and human, with different pupil sizes and focal lengths (Rochester). This device will also be used in collaboration with John Flannery's group at UCB.
2. An AOSLO that tracks the pupil and relaxes the requirement for head stability in patients (Berkeley). This instrument incorporates software corrections for image distortions caused by eye movements.
3. An AOSLO for clinical researchers at DEI/USC. This device will separate the light sources required to correct the eye's aberrations and that required for retinal imaging, to maximize both the quality of the correction and the quality of the retinal image (LLNL).
4. An AOSLO that will incorporate heterodyne detection, polarimetry, and retinal tracking to increase system performance (IU). Retinal tracking is under development through a subcontract with Physical Sciences, Inc.

STATUS OF RESEARCH AND PARTNERSHIP
Functional instruments capable of collecting retinal images at high magnification have now been demonstrated at 3 of the 4 sites originally contracted to build instruments (Rochester, Berkeley, and IU), with the fourth site (LLNL) planning completion within the next two months. The rapid progress we have achieved so far can be partly attributed to extensive collaboration between investigators from the six participating institutions. All four instruments share a common core design that has been developed through six committees, each tasked with making design recommendations for a different subsystem in the instrument. Inter-institutional collaboration is facilitated by video/teleconferencing as well as face to face meetings.

Instrumentation Highlights
Mitigating against eye movements. Through work in Roorda's lab, and subcontracts to Montana State University and to Physical Sciences Inc., the BRP has made important progress toward the goal of tracking the retina and removing the artifacts in high resolution images that are caused by eye movements. Incidentally, a byproduct of image stabilization is that the technology has produced the most accurate eye movements recordings ever made.
MEMS (MicroElectrical Mechanical Systems) deformable mirrors. A major concern in the development of our proposed AO instruments has been the availability of suitable deformable mirrors. The BRP continues to capitalize on the efforts of the Center for Adaptive Optics (CfAO), an NSF-funded

Science and Technology Center, to work with companies to develop a new kind of deformable mirror for vision science applications. The BRP decided to move forward with Boston Micromachines Corp, which recently produced the first MEMS mirrors that meet the minimum requirements for vision science applications.

Solutions to extend deformable mirror dynamic range. The BRP is also investigating novel solutions to increase the dynamic range of MEMS mirrors, which include reflecting the light twice off a single mirror and using a cascade of two different mirrors in a concept analogous to the use of a woofer and tweeter in loudspeakers.

Scientific Highlights

Imaging ganglion cells in vivo. The Rochester group has demonstrated that it is possible to image ganglion cells using fluorescence imaging in the living primate retina. We anticipate far more detail when the high resolution capability of the FAOSLO are fully implemented.

Imaging flow of single blood cells in vivo. Roorda's laboratory (Berkeley) continues to develop methods to measure leukocyte velocity and pulsatility near the fovea as well as methods to determine axial locations of blood vessels in the retina.

High resolution imaging of the lamina cribrosa in glaucoma. Roorda's laboratory obtained high contrast, high resolution images of the lamina cribrosa in a monkey model for glaucoma.

Imaging in vivo retinal features that may be single RPE cells. IU have identified features in there AOSLO images that may represent individual RPE cells. This capability could have important implications for monitoring changes in the RPE at a microscopic spatial scale in vivo.

New fluorescent markers. John Flannery at UC, Berkeley has been developing an impressive arsenal of fluorescent markers to stain four different classes of retinal cells.

Significance

The Bioengineering Partnership has made significant progress in developing four instruments that allow noninvasive microscopic imaging of the living retina, each optimized to have different capabilities, such as fluorescence or polarization imaging. These instruments allow us to examine retinal disease at the cellular level in living eyes, something that before now generally required microscopic examination of post mortem tissue. The technology facilitates new studies of normal retina, continuous monitoring of disease progression in abnormal retina, and evaluation of the efficacy of retinal therapies.

ISSUES

Two key personnel have moved into new institutions in the last year. Burns and Elsner moved to Indiana University and Roorda moved to UC Berkeley. Roorda is already settled in Berkeley and all BRP-supported adaptive optics systems are up and running. Burns and Elsner are currently in the process of moving and expect no serious setbacks.

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PROJECT TITLE: General Purpose Brain-Computer Interface (BCI) System

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GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke and National Institute of Biomedical Imaging and Bioengineering

ABSTRACT
Brain-computer interfaces (BCIs) give people with severe motor disabilities (e.g., amyotrophic lateral sclerosis (ALS), brainstem stroke, cerebral palsy, and spinal cord injury) communication and control technology that does not depend on neuromuscular output. BCIs can allow people who are severely paralyzed to express wishes to caregivers, use word processing programs, access the Internet, or even operate neuroprostheses.

Up to now, BCI research has demonstrated that a variety of different methods using different brain signals, signal analyses, and operating formats can convey a person's commands to a computer. Future progress that moves from this demonstration stage to practical applications of long-term value to those with motor disabilities requires a flexible general purpose BCI system that can incorporate, compare, and (if indicated) combine these different methods, and can support generation of standard protocols for the clinical application of this new communication and control technology. The development and clinical validation of a general purpose BCI system is the goal of this Bioengineering Research Partnership (BRP) application.

The investigators in this partnership have been in the forefront of research into current BCI methods, and together they have extensive experience in the development of BCI systems. The aims of this proposal are: (1) to develop a flexible general purpose BCI system that can incorporate any of the relevant signals, analyses, and operating formats and can be configured for laboratory or clinical needs; (2) to use the system to compare, contrast, and combine relevant brain signals and signal processing options during BCI operation and thereby develop a standard protocol for applying BCI technology to the needs of individual users; (3) to apply the system and protocol to address specific communication needs of people with severe motor disabilities and show that BCI technology is both useful to and actually used by these individuals; (4) to apply the system and protocol to develop the use of neuronal activity or field potentials recorded within or on the cortex for communication and control, and to define the relationships between these signals and scalp-recorded signals that might be used to guide or supplement invasive methods.

Achievement of these aims and dissemination of the resulting technology to other research groups should advance BCI research from its current stage of laboratory demonstrations to development and validation of a general purpose BCI communication and control technology that can incorporate all relevant brain signals and has clear practical value for those with motor disabilities.

STATUS OF RESEARCH AND PARTNERSHIP

The past year has seen substantial progress in a number of areas. The program has focused on the development and dissemination of BCI2000 and the use of this system to train people with and without disabilities to use a variety of BCI methods including sensorimotor (i.e., mu and beta) rhythms and the P300 evoked potentials.

Current efforts are concentrated on the following objectives:

1. To continue development and dissemination of BCI2000, the general-purpose BCI software system that readily accommodates different signals, recording hardware, signal analysis and translation algorithms, and applications. The system has been provided free of charge to about 50 research labs, is being supported by a very active website, and is being used in a wide variety of studies. BCI2000 now includes tools for both on- and off-line use, e.g., SIGFRIED (SIGnal modelling, for Identification and Event Detection), a non-hypothesis driven screening tool and MARIO (in conjunction with Febo Cincotti), a free-standing tool for offline analysis. In June 2005, we planned and hosted the first BCI2000 training workshop. It attracted 41 participants.

2. To further improve EEG signal analysis and translation algorithms. We are developing user-specific methods for sensorimotor rhythm analysis. We are also exploring adaptive translation algorithms that continually adjust the recording locations and frequency bands used for control; and we are developing real-time non-hypothesis-driven methods for identifying and localizing useful brain signals.

3. To further develop EEG-based multidimensional and sequential movement control. We are improving two-dimensional cursor control, and adding a select function, so that users can move a cursor (or a robotic arm) to a location and select (or grasp) the object located there. We are also developing a three-dimensional protocol.

4. To develop applications using other EEG features, such as P300 and SSVEPs. Recent advances in P300 signal analysis and translation are substantially improving the speed and accuracy of matrix-based spelling.

5. With our partners at Washington University in St. Louis and the University of Washington in Seattle, to explore BCI applications using electrocorticographic (ECoG) activity recorded from the cortical surface. We study people implanted with cortical surface arrays for short periods prior to epilepsy surgery. The higher amplitude, topographical resolution, frequency range, and signal-to-noise ratio of ECoG (compared to EEG) suggest that it may be extremely useful for BCI applications. Early results show 2-D control.

6. With our Tűbingen Partner, to continue to study the use of different BCI methods by people with amyotrophic lateral sclerosis (ALS) and other severe motor disabilities. This work uses a simplified and easily portable BCI2000-based system and concentrates on studying people in their homes. It incorporates standardized methods for assessing quality of life.

7. With our Atlanta partner, to continue to work on BCI2000 software adaptations including work with the P300 program, a 3-D sensorimotor application, and a SSVEP protocol.

ISSUES

Specific Aim 1. We are continuing to develop methods for incorporating other signals into BCI2000 and add additional offline data analysis options. We are developing a user-friendly stand-alone clinical version of BCI2000 and adding user applications. We also plan to explore additional feature extraction methods for neuronal activity and incorporate additional feedback modalities into user application modules. We will continue to offer BCI2000 to other research groups with full documentation and continuing consultation, and provide additions and modifications as needed by Aim 3. Plans are underway to develop BCI2000 in conjunction with laboratories studying stroke and spinal cord injury rehabilitation.

Specific Aim 2. We are continuing within-subject method comparison experiments and are incorporating results into the clinical protocols of Aim 3.

Specific Aim 3. We are continuing to recruit new users and are incorporating these Aim-1 and Aim-2 results into the user training protocols. We plan to continue support to BCI users who wish to continue BCI use. In the coming months, BCI applications will be developed for specific consumers and telemonitoring will be used to follow daily use. We plan to assess quantitatively extent of BCI use and its value to the user.

Specific Aim 4. We are continuing to improve algorithms for translating electrocorticographic (ECoG) activity into cursor control and evaluate results in standard fashion. We plan to continue to define relationships between electrocorticographic (ECoG) activity and signals recorded from scalp.

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PROJECT TITLE: Understanding/Improving Flow Dynamics in Fontan Surgeries

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute

ABSTRACT

The overarching hypothesis is that a fundamental understanding of the single ventricle and associated total cavopulmonary (TCPC) anatomy and physiology will lead to improved surgical planning, design and potential for improved long term patient outcomes. To address this, we propose the investigation of the following specific aims: (1): Qualitative and quantitative assessment of Fontan flow dynamics for various TCPC anatomic geometries and physiologic conditions in order to establish optimal TCPC anatomic templates, (2): Evaluate the impact of different source materials used in the IVC to RPA connection (during the TCPC surgery), on the local flow dynamics, (3) Establish an anatomic and materials database that would be used to validate computationally based surgical planning and design, (4): Provide information and computational simulations to optimize the TCPC in an individual patient, (5): Investigate the effects of ascending aorta flow dynamics on the energetics of the Fontan connection, (6): Explore the feasibility of using a "pressure regulator/pump" to help reduce central venous pressure in Fontan patients and thus potentially improve their long term outcome and quality of life.

STATUS OF RESEARCH AND PARTNERSHIP

Studies at Georgia Tech have focused primarily on the development and validation of the computational (CFD) and experimental fluid dynamics analysis methods. CFD simulations provide a full 3-D description of the flow field, enabling a fundamental understanding of the complex TCPC hemodynamics. However, prior to formulation of any conclusions and determining surgical relevance we feel it is critical to ensure reliability of our CFD results. Validation of our computational simulations against their experimental counterparts is integral to our methodology and has been carried on several different TCPC templates this year. This CFD validation campaign included both idealized and anatomic geometries. CFD runs have been done on 8 anatomic models (5 TCPC and 3 bidirectional Glenns). Extensive studies have been conducted on the simulation of transient behavior of flows in the TCPC models using the new Beowulf computer cluster. It is acknowledged that the attempts to simulate 3-D highly unsteady flows using most commercial flow solvers failed to obtain conclusive results with practical time step and mesh resolution. In order to circumvent this problem, work is being done on developing an in house CFD code that would successfully capture unsteadiness associated with the complicated Fontan geometries. Work is also being done to understand the effects of different parameters on these TCPC models such as fenestration, LPA stenosis, varied lung resistance, and the role of ventricular assist devices (VAD) using the lumped parameter model.

In the experimental studies thus far, 7 anatomic models have been studied. These models consisted of 3 extra cardiac and 4 intra atrial TCPCs. Experimental studies were performed using rapid prototype models created from three dimensional anatomic reconstructions of the TCPC using axial Magnetic Resonance Images. Power Loss has been computed for each of these models and the results have shown the importance of pulmonary artery diameter in the efficiency of the TCPC. Three dimensional Particle Image Velocimetry (PIV), and Magnetic Resonance Phase Contrast Velocimetry (PC MRI) are also being

performed on these models in order to obtain qualitative and quantitative flow structures. The results are being compared to those obtained from CFD for validation.

Progress has been significant with regards to the in vivo lamb studies performed last year. Numerous steps have been taken in order to improve the success rate of Fontan animal studies. Donor animals for blood are no longer required reducing the number of animals by one half. The main obstacle limiting lamb survival was increased pulmonary vascular resistance after the Fontan circulation had been created. In order to prevent fluid in the lungs and other complications, the lungs were ventilated while on bypass. Since these changes were incorporated, a total of six successful lamb studies with a creation of a total cavopulmonary extra-cardiac Fontan circulation have been performed.

The Children's Healthcare of Atlanta (CHOA), the Children's Hospital of Philadelphia (CHOP), and UNC each transferred MRI patient data sets to a secure server at Georgia Tech. Currently; the patient database contains MRI (anatomy and flow) information on 100 TCPC patients. The mean age is about 11 years and there is a 1.5:1 ratio of males to females. Most patients are non-Hispanic Caucasians, and intra-atrial connections dominate the patient population. Within the data sets all five templates of the TCPC are represented. However, the interrupted IVC and the IVC-MPA connection were limited because of very few cases of heterotaxy syndrome and because only one surgeon performed the IVC-MPA connection.

Axial MR anatomy images are obtained via balanced fast gradient echo sequences. In order to create data sets composed of isotropic voxels, which are better suited for reconstruction, a technique called adaptive control grid interpolation (ACGI) is used to enhance through-plane spatial resolution. Each TCPC is isolated within the enhanced MR data using a shape-element segmentation technique. Intensity thresholding and edge detection methods are used to create a scaffold around the TCPC, within which the vascular area of interest is defined by the motion of a shape element. Computer-aided design tools are used to produce a 3-D model of the TCPC from the segmented data to be used in rapid prototyping and CFD.

In Matlab, a program called Flowfinder was written to analyze the velocity images. The program calculates mean flow for the entire cardiac cycle, mean flow for each phase in the cardiac cycle, maximum velocity in each phase of the cardiac cycle, mean velocity during the cardiac cycle, and area of the vessel lumen. The velocities extracted by Flowfinder can then be used as boundary conditions for the CFD simulations. Flow data is available for 97 / 100 patients in the database. Phase Contrast MRI slices are obtained at each of the vessels associated with the TCPC (SVC, IVC, LPA, and RPA). The vessels are segmented using a semi automatic scheme and flow parameters are computed. In some patients we also have slices through the ascending aorta that can be used to compute the total cardiac output, and the input cardiac power computed using the cuff pressure. These studies help us in evaluating the significance of the power loss seen in the TCPC.

ISSUES

The investigators and research staff at CHOA, CHOP, and UNC will continue to work diligently to enroll minority and female subjects into the above referenced study. We are, however, very cognizant of the fact that our patient population is, for the most part, white males.

We are also experiencing difficulties in obtaining a contiguous 3 dimensional stack of PC MRI with encodings in all three directions through the entire TCPC. Due to strict time limitations associated with obtaining PC MRI we acquire in vivo velocity data only at the four vessels. We are looking into new sequences that would help in speed up the process of PC MRI acquisition which would provide a 3 dimensional flow field that could be used for accurately identifying in vivo flow structures and for comparison with those obtained experimentally and computationally.

Coming into the fourth year of the project, it has been a challenge to get sufficient surgeon participation towards this study. Since the surgeons are going to benefit the most from this study it is imperative to get their active input for making further headways in the project.

Though the initial aim of the reconstruction database was not to generate a detailed clinical study, the quality, volume of data, and 3D visual access to the anatomic information have incited several interesting studies.

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PROJECT TITLE: Robot-Assisted Platform for Intratumoral Delivery

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ABSTRACT
Intratumoral therapies of prostate cancer include the delivery of brachytherapy or ablation energy sources, and adenoviral injection, through a minimally invasive, transperineal approach. They require quantitative, optimized treatment planning, precise placement of the needles/probes according to the treatment plan, and real time dosimetric evaluation in the operating room as deviations from the treatment plan are detected. Recent studies suggest that imprecisions in standard brachytherapy using the manual technique cause higher than previously-appreciated complication rates, and may be the cause of local failure in 15% of the patients.

The major goal of the proposed work is to develop the Robot-Assisted Platform for Intratumoral Delivery (RAPID) for integrated treatment of prostate cancer, and to demonstrate the safety, efficacy and clinical effectiveness through bench/phantom tests and a Phase I clinical study. A number of maturing component technologies previously developed by the bioengineering research partners will be combined in this major collaboration, including the first robotic system for urological applications with early experience on actual patient treatment, the first treatment planning system with intraoperative dosimetry optimization, and the first needle/probe tracking system for real time ultrasound-based treatment verification, permitting re-planning and re-optimization of therapy delivery. The integrated RAPID system, designed based on prototype subsystems developed at each of the research partners, will initially focus on interstitial brachytherapy of prostate cancer. It is aimed at delivering precise, non-coplanar 3D conformal radiation rapidly and with assured consistency, and to incorporate such complex concurrent therapies as radiosensitization and mixed agent/strengths brachytherapy. Primary outcome variables including implant quality, cost, morbidity and learning curve will be examined under the clinical study by comparison with historical controls.

The long-term objective of the RAPID project is to incorporate the delivery of concomitant therapeutic agents intratumorally for cancer in the prostate as well as in other organ systems. The multi-agent, multi-modality capabilities of the RAPID system will be continually exploited towards total optimization of a turnkey in vivo diagnosis and therapy engine for localized cancers of solid organs.

STATUS OF RESEARCH AND PARTNERSHIP
During the second grant year of this BRP project, the following key studies have been accomplished: (1) robotic design of needling mechanism, ultrasound probe mechanism and 6 DOF robotic frame, (2) software implementation of user interface including imaging, 3D anatomy modeling and control interface with hardware, (3) dosimetry inverse planning engine modification to permit both rectilinear and angulated needle approaches, (4) inverse re-planning engine for dynamic dosimetry update. Fabrication of the hardware system is underway. In addition, extensive bench experiments were conducted to investigate and optimize needle insertion techniques, including measurement of force, velocity modulation, needle oscillation and rotation during incursion. It was also found necessary to conduct a clinical study to

measure the actual force pattern and velocity profile of needle incursion by the surgeon during actual prostate brachytherapy procedures on patients. This study was completed in the current grant year under an IRB-approved protocol and informed consent.

The ultrasound probe mechanism permits two motions: linear translation and rotation. Encoders are used to feedback the real-time translation distance and rotation angle, which are important for image guidance and verification such as detection of implanted seeds. The linear translation and rotation can also be actuated by hand in manual mode, which permits clinician intervention to the robotic procedure.

The needling mechanism actuates the central stylet (core) and outer cannula (sheath) of the needle separately. Two ball screws driven by two servo DC motors are used to drive the stylet translation motion and cannula translation motion. The bevel tipped cannula will rotate while the needle is inserted into tissue, thus reducing tissue deformation and needle deflection. Two Z-directional force sensors and one X-Y sensors are installed at the ends of the stylet and the cannula and in the front end of the mechanism, to detect the needle's loads along the axial direction and bending force of the cannula. The design of the needling mechanism contains full provisions for maintaining sterility per requirement specification.

A large number of laboratory experiments were conducted in this grant year on the testbed robot built in Grant Year 1, on characterizing and optimizing needle incursion strategies to precisely reach a target in tissue. We also found it necessary to design a clinical study to collect in-vivo data from 10 patients undergoing prostate brachytherapy, in order to gain data on the actual force, velocity, acceleration patterns experienced by the surgeon during needle insertion into patients. No such data had been reported at all in the literature. The study protocol was duly approved by the institutional IRB and was carried out with informed consent. To briefly summarize: The average maximum forces outside and inside the prostate for 18 Ga needle are similar, 7.53N and 6.97N, respectively (for patient #1-5), but for 17 Ga needle these forces are quite different, 15.03N and 7.11N. The Fz forces for 17 Ga needle before entering into the prostate is about 7N higher than that of 18 Ga needle, but the forces when the needles are in the prostate are similar. This information is crucial for design guidance for achieving a clinically acceptable system.

The brachytherapy inverse planning engine developed in Year 1 incorporating rectilinear and conical dosimetry has been extended to plan the delivery of hybrid rectilinear and angulated needles and to perform dynamic replanning by accounting for unanticipated displacement of radioactive seeds in a portion of the implant. Hybrid implantation is designed to overcome pubic arch interference and to extend the coverage region of the robot beyond the space constraints presented in the operating room. Dynamic replanning will allow the brachytherapist to compensate for deficiencies in dose coverage when some needles and/or seeds are placed with inaccuracy due to unavoidable factors such as edema and tissue deformation.

ISSUES

There is no major issue at this time.