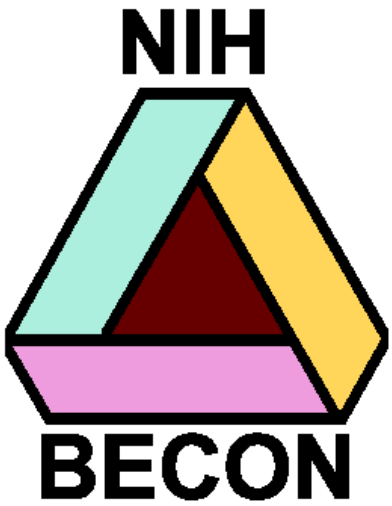
SIXTH ANNUAL BIOENGINEERING RESEARCH PARTNERSHIP GRANTEE MEETING



National Institutes of Health
Bioengineering Consortium

Bethesda North Marriott Hotel
& Conference Center
Bethesda, Maryland
July 13-14, 2006

Bioengineering Consortium

National Institutes of Health
Bethesda, MD 20892

Welcome to the Sixth Annual Bioengineering Research Partnership Grantee Meeting.

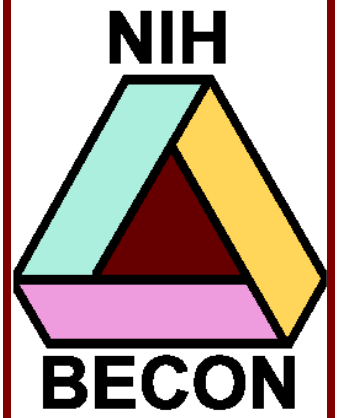
This February marked the eighth anniversary of the Bioengineering Consortium (BECON),which provides a focus for trans-Institute biomedical engineering research and training activities at the National Institutes of Health (NIH). Active participation by almost all NIH Institutes and Centers and other Federal agencies has enabled the realization of significant advances in basic knowledge and health care by applying engineering, physical, and computational science principles and techniques to addressing problems in biology and medicine. Appropriately, the Consortium is administered by the National Institute of Biomedical Imaging and Bioengineering, which is committed to integrating the physical and engineering sciences with the life sciences to advance basic research and health care.

In support of its mission, the BECON has coordinated several trans-NIH initiatives aimed at encouraging and supporting multidisciplinary and integrative approaches to biomedical research and training. One of the most successful and visible of these initiatives is the Bioengineering Research Partnership (BRP) Program, which was first announced in October 1999. The partnerships that have developed in response to this program are examples of the types of collaborations among scientific disciplines and organizations that can provide significant advances in health care and fundamental understanding. To date, more than 170 BRP awards have been made by 16 NIH research Institutes and Centers, for a total investment of more than $800 million.

This meeting is the sixth time that BRP grantees, BECON members, and NIH Institute and Center representatives will gather to discuss research projects, bioengineering issues, and the program in general. This is the second meeting in which all grantees will present posters and more than one researcher can represent each project. This meeting is also a milestone in that some grants have successfully undergone competitive renewal. As always, your perspectives and suggestions concerning the program in general, partnership experiences, and bioengineering research and training needs are welcome and solicited. Also, please take this opportunity to meet with your NIH Institute/Center project manager to discuss progress and concerns associated with your grant.

I hope that the BRP Grantee Meeting is valuable and enjoyable to you as it has been in past years. All the BECON members and the NIH program staff look forward to your participation and comments.

Michael F. Huerta, Ph.D., Chair
Bioengineering Consortium



Agenda

SIXTH BIOENGINEERING RESEARCH PARTNERSHIP
GRANTEE MEETING

July 13-14, 2006
Bethesda North Marriott Hotel and Conference Center
Bethesda, Maryland

AGENDA

Thursday, July 13

7:45 AM	**Registration and Poster Setup**
8:15	**Welcome and Orientation**
8:30	**NIH Updates** Current Issues at NIH – *Norka Ruiz-Bravo (OER)* BRP Evaluation – *Martha Lundberg (NHLBI)* NIBIB Status and Directions – *William Heetderks (NIBIB)*
10:00	**Break**
10:30	**Grantee Presentations – Basic Research** *Moderator: Theresa Smith (NIBIB)*
12:30 PM	**Lunch**
1:30	**Poster Session- I**
3:00	**Grantee Presentations – Translational Research** *Moderator: Todd Merchak (NIBIB)*
5:00	**Discussion, Poster Viewing, and Reception**
6:00	**Adjourn for the day**

Friday, July 14

8:00 AM	**Registration**
8:30	**Grantee Presentations – Applied Research** *Moderator: Elijah Weisberg (NIBIB)*
10:30	**Break and Poster Viewing - II**
11:30	**Discussion**
Noon	**Adjourn meeting**

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GRANTEE ORAL PRESENTATIONS

Basic Research (Thursday, 10:30 AM - 12:30 PM)

Moderator: *Theresa Smith (NIBIB)*

Nicholas Abbott (NCI) – Biomolecular Analysis using Liquid Crystals
Gary Brittenham (NIDDK) – High Tc Susceptometer for Magnetic Measure of Body Iron
Francesco Cerrina (NHGRI) – Light-directed Synthesis of Genes & Other Biomolecules
Jeffrey Fredberg (NHLBI) – Micromechanics of the Airway Smooth Muscle Cell
Lee Miller (NINDS) – Development of a Bidirectional Brain Machine Interface
John Pearson (NIGMS) – Multi-scale Observation and Modeling of IP3/Ca Signaling
Wendy Suzuki (for Emery Brown) (NIDA) – Dynamic Signal Processing Analyses of Neural Plasticity
Matt Prasad (for Brent French) (NHLBI) – Gene Therapy for Myocardial Stunning and Infarction
Angelique Louie (for Russell Jacobs) (NIBIB) – Multimodal mPET & mMRI Imaging Instrumentation

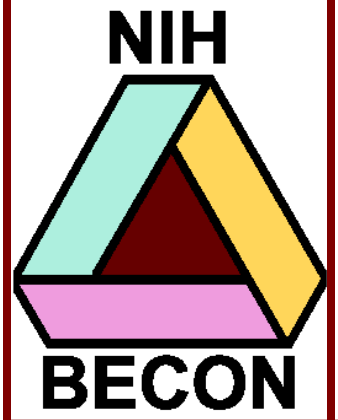
Translational Research (Thursday, 3:00 PM – 5:00 PM)

Moderator: *Todd Merchak (NIBIB)*

Colleen Flanagan (for Scott Hollister) (NIDCR) – Engineering Joint Scaffolds for Function/Regeneration
E. Antonio Chiocca (NCI) – Interdisciplinary Tumor Complexity Modeling
Edward Crandall (NHLBI) – Absorption Mechanisms for Peptide/Protein Drugs via Lung
Kevin Gillis (NINDS) – Microchip Devices to Assay Quantal Exocytosis
Robert Gilmour (NHLBI) – MEMS Sensors for Arrhythmia Detection and Interventions
Richard Rabbitt (NIDCD) – Micro-Electric Impedance Spectroscopy of Hair Cells
Mehmet Toner (NIGMS) – Cellular Engineering for Metabolic Stasis
Enrique Izaguirre (for Bruce Hasegawa) (NIBIB) – Imaging Structure and Function in Small Animals
Francois Berthiaume (for Martin Yarmush) (NIAID) – Living Cell Arrays for Real Time Functional Genomics

Applied Research (Friday, 8:30 AM – 10:30 AM)
Moderator: *Elijah Weisberg (NIBIB)*

Brian Litt (for Marc Dichter) (NINDS) – An Implantable Device to Predict and Prevent Seizures
John Frangos (NIBIB) – Anti-Inflamatory Coatings for Biomaterials
Eric Hoffman (NHLBI) – Image and Model Based Analysis of Lung Diseases
Tuan Vo Dinh (NCI) – Advanced Multispectral Imaging for Medical Diagnostics
Richard Weir (NIBIB) – Multifunction Prosthesis Control using Implanted Sensors
Robert Bartlett (NHLBI) – Development of a Total Artificial Lung
Kai Thomenius (for Paul Carson) (NCI) – Combined Digital X-Ray and Ultrasound Breast Imaging
Jian Zhang (for Mark Clemens) (NIDDK) – Engineering Aspects of Liver Support Systems
John Frangos (for Marcos Intaglietta) (NHLBI) – Bioengineering Design of Artificial Blood
Don Miller (for John Werner) (NEI) – Ophthalmic Imaging using Adaptive Optics and OCT
David Worchester (for Stephen White) (NCRR) – Cold Neutrons for Biology and Technology



Poster Assignments

SIXTH BIOENGINEERING RESEARCH PARTNERSHIP
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POSTER ASSIGNMENTS

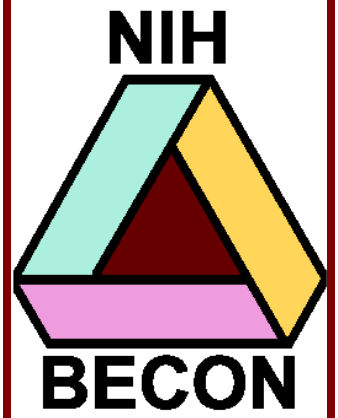
Poster #	PI Name	Project Title	Speaker Session	Funding Institute
1	**ABBOTT, NICHOLAS L**	Biomolecular Analysis using Liquid Crystals	**BA**	NCI
2	**BARTLETT, ROBERT H.**	Development of a Total Artificial Lung	**AP**	NHLBI
3	**BOONE, JOHN M**	Breast CT Scanner for Earlier Cancer Detection		NIBIB
4	**BORENSTEIN, JEFFREY T**	Micromechanical Device for Intracochlear Drug Delivery		NIDCD
5	**BRITTENHAM, GARY M**	High Tc Susceptometer for Magnetic Measure of Body Iron	**BA**	NIDDK
6	**BROWN, EMERY NEAL**	Dynamic Signal Processing Analyses of Neural Plasticity	**BA**	NIDA
7	**BROWN, THOMAS DUDLEY**	Wear Analysis of Intervertebral Total Disc Replacements		NIAMS
8	**BUCHANAN, THOMAS S**	FES and Biomechanics: Treating Movement Disorders		NICHD
9	**CARSON, PAUL L**	Combined Digital X-Ray and Ultrasound Breast Imaging	**AP**	NCI
10	**CERRINA, FRANCESCO**	Light-directed Synthesis of Genes & Other Biomolecules	**BA**	NHGRI
11	**CHEN, ZHONGPING**	Optical Biopsy Using MEMs Technology		NCI
12	**CHIEN, SHU**	Mechanical and Molecular Bases of Endothelial Remodeling		NHLBI
13	**CHIOCCA, ENNIO ANTONIO**	Interdisciplinary Tumor Complexity Modeling	**TR**	NCI
14	**CHURCHILL, BERNARD M**	Uropathogen Detection Using DNA Biosensors		NIBIB
15	**CLARKE, ROBERT R.**	Molecular Analysis of Human Breast Cancer		NCI
16	**CLEMENS, MARK G**	Engineering Aspects of Liver Support Systems	**AP**	NIDDK
17	**CORLEY, RICHARD A**	3D Imaging & Computer Model of the Respiratory Tract		NHLBI
18	**CRANDALL, EDWARD D**	Absorption Mechanisms for Peptide/protein Drugs via Lung	**TR**	NHLBI
19	**DALE, ANDERS M**	Spatiotemporal Brain Imaging: Microscopic & System Level		NIBIB

20	**DAVIES, PETER F**	Cell and Molecular Studies in Cardiovascular Engineering		NHLBI
21	**DEGRADO, WILLIAM F**	Proteomics to Biomimetic Polymers: Engineering Principle		NIBIB
22	**DEL NIDO, PEDRO J.**	Image-guided Intracardiac Beating Heart Surgery		NHLBI
23	**DEVOE, DONALD L**	Proteomics of Cell Death via 2-D Microfluidic Profiling		NIGMS
24	**DEWEERTH, STEPHEN P**	A 3-D Microfluidic/Electronic Neural-Interfacing System		NIBIB
25	**DICHTER, MARC A**	An Implantable Device to predict and Prevent Seizures	**AP**	NINDS
26	**DORDICK, JONATHAN S.**	High-Throughput Solid-Phase Combinatorial Biocatalysis		NIGMS
27	**DOYLE, MARK**	Rapid Flow Evaluaton by Magnetic Resonance Imaging		NHLBI
28	**DUNCAN, JAMES S**	Bioimaging and Intervention in Neocortical Epilepsy		NIBIB
29	**EATON, GARETH R**	In Vivo EPR Bioengineering Research Partnership		NIBIB
30	**EDGERTON, V. REGGIE**	Robotically Generated Locomotion in Rodents		NIBIB
31	**FELD, MICHAEL S**	Spectroscopic Imaging and Diagnosis of Neoplasia		NCI
32	**FELEPPA, ERNEST JOSEPH**	Integrated Ultrasonic Systems for Noninvasive Therapy		NCI
33	**FERRARA, KATHY**	Ultrasound Imaging and Local Drug Delivery in Tumors		NCI
34	**FRANGIONI, JOHN V**	Intraoperative Near-Infrared Fluorescence Imaging		NCI
35	**FRANGOS, JOHN A**	Anti-Inflamatory Coatings for Biomaterials	**AP**	NIBIB
36	**FREDBERG, JEFFREY J**	Micromechanics of the Airway Smooth Muscle Cell	**BA**	NHLBI
37	**FRENCH, BRENT A**	Gene Therapy for Myocardial Stunning and Infarction	**BA**	NHLBI
38	**FUKAMACHI, KIYOTAKA**	Development and Clinical Testing of CorAide RVAD/BVAD		NHLBI
39	**GILBERT, CHARLES D**	Molecular Analysis of Visual Processing		NEI
40	**GILLIS, KEVIN D**	Microchip Devices to Assay Quantal Exocytosis	**TR**	NINDS
41	**GILMOUR, ROBERT F**	MEMS Sensors for Arrhythmia Detection and Interventions	**TR**	NHLBI
42	**GREENBERG, ROBERT JAY**	Development/Testing of Artifical Retinas for the Blind		NEI
43	**GRODZINSKY, ALAN J**	Self-Assembling Peptides for Tissue Engineering		NIBIB
44	**HALPERIN, HENRY R**	Magnetic Resonance Guided Electrophysiology Intervention		NHLBI
45	**HASEGAWA, BRUCE H.**	Imaging Structure and Function in Small Animals	**TR**	NIBIB
46	**HIRSCHI, KAREN**	Tissue Engineering of Hematopoietic Bone		NIBIB

47	**HOFFMAN, ERIC A**	Image and Model Based Analysis of Lung Diseases	**AP**	NHLBI
48	**HOLLISTER, SCOTT J**	Engineering Joint Scaffolds for Function/Regeneration	**TR**	NIDCR
49	**HOUCK, RAYMOND K**	Quantitation of Cellular Protein Production in Real Time		NIBIB
50	**HUMPHREY, JAY D**	Histo-Mechanics & Biology of Remodeling in Hypertension		NHLBI
51	**INTAGLIETTA, MARCOS**	Bioengineering Design of Artificial Blood	**AP**	NHLBI
52	**JACOBS, RUSSELL E**	Multimodal mPET & mMRI Imaging Instrumentation	**BA**	NIBIB
53	**JAIN, RAKESH K.**	Integrative Biology of Tumor Metastasis		NCI
54	**JOHNSON, JOHN E**	Plant Viruses as Platforms for Biomaterials		NIBIB
55	**KAYE, JEFFREY A**	Intelligent Systems for Detection of Aging Changes		NIA
56	**KELLUM, JOHN A**	Systems Engineering of Pheresis Intervention for Sepsis		NHLBI
57	**KIRSCH, WOLFF M.**	Iron Metabolism Alterations in Alzheimer's Disease		NIA
58	**KLEINFELD, DAVID**	Manipulating Neural Tissue With Ultra-Short Laser Pulses		NIBIB
59	**LEMONS, JACK E**	Analyses of InSitu and Explanted Surgical Implant Device		NIBIB
60	**LI, S KEVIN**	Methods & Noninvasive PK Study to Improve Iontophoresis		NEI
61	**LI, SHU-TUNG T**	Type 1 Collagen-Based Nerve Guide for PNS Regeneration	**TR**	NICHD
62	**LING, CLIFTON C**	Multimodality Biological Imaging of Cancer/Tumor Hypoxia		NEI
63	**LOEB, GERALD E**	BION Treatment of Neuromuscular Dysfunction		NCI
64	**MAITLAND, DUNCAN J**	Shape Memory Polymer Devices for Treating Stroke		NIBIB
65	**MARK, ROGER G**	Integrating Data, Models, and Reasoning in Critical Care		NIBIB
66	**MATTHEWS, MICHAEL A.**	Processing of Materials for Improved Biocompatibility		NIBIB
67	**MAUDSLEY, ANDREW A.**	Partnership for MR Spectroscopic Imaging Data Processing		NIBIB
68	**MC INTIRE, LARRY V.**	Leukocyte Trafficking: From Flow Blood to Tissue		NHLBI
69	**MCKNIGHT, TIMOTHY E**	Nano Arrays for Real-Time Probing Within Living Cells		NIBIB
70	**MEANEY, DAVID F**	Force Transmission in the Central Nervous System		NICHD
71	**MILES, RONALD N**	Sensing and Processing for Directional Hearing Aids		NIDCD
72	**MILLER, LEE E**	Development of a Bidirectional Brain Machine Interface	**BA**	NINDS
73	**MUEHLEMAN, CAROL**	Novel X-Ray Technology for Degenerative Joint Diseases		NIAMS

74	**NARAYANA, PONNADA A**	MR Image Analysis in MS: Identification of a Surrogate		NIBIB
75	**NIE, SHUMING**	Nanotechnology Linking Biomarkers with Cancer Behavior		NCI
76	**NOLAN, JOHN P**	Raman Flow Cytometry for Diagnostics and Drug Discovery		NIBIB
77	**OLSEN, DON B**	New Magnetically Suspended LEV-VAD		NHLBI
78	**PEARSON, JOHN E**	Multi-scale Observation and Modeling of IP3/Ca Signaling	**BA**	NIGMS
79	**PECKHAM, P. HUNTER**	Development of Networked Implantable Neuroprostheses		NIBIB
80	**PERTSOV, ARKADY M**	3D Imaging of Electrical Activity in Myocardial Tissue		NHLBI
81	**PIANETTA, PIERO A**	Multi-keV X-ray Microscopy Facility for Bio-imaging		NIBIB
82	**RABBITT, RICHARD D**	Micro-Electric Impedance Spectroscopy of Hair Cells	**TR**	NIDCD
83	**RAMSEY, JOHN M**	Nanotechnology for the Structural Interrogation of DNA		NHGRI
84	**RENSHAW, PERRY F**	High Field MR Research in Drug Abuse		NIDA
85	**RICHARDS-KORTUM, REBECCA RAE**	Optical Systems for In Vivo Molecular Imaging of Cancer		NCI
86	**RYBAK, ILYA A**	Spinal Control of Locomotion: Studies and Applications		NINDS
87	**SAHN, DAVID J.**	High Frequency Ultrasound Arrays : Intracardiac Imaging		NHLBI
88	**SALAMA, GUY**	High-Speed, Depth-Resolved Images of Cardiac physiology		NHLBI
89	**SHAPIRO, IRVING M**	Smart Substrates for a New Generation of Implants		NIAMS
90	**SKALAK, THOMAS C**	Integrated Control of Vascular Pattern Formation		NHLBI
91	**SMITH, DESMOND J**	Transcriptome and Proteome Mapping of the Mouse Brain		NS
92	**SMITH, MICHAEL B**	High Field MRI: Limitations and Solutions		NIBIB
93	**SMITH, WILLIAM A**	Magscrew TAH Testing thru Pre-Clinical Readiness		NHLBI
94	**SOKURENKO, EVGENI V**	Dynamic Properties of Bacterial Adhesions		NIBIB
95	**STUPP, SAMUEL I**	Regenerative Scaffold Technologies for CNS and Diabetes		NIAID
96	**TOMSIA, ANTONI P**	Complex Nanocomposites for Bone Regeneration		NIDCR
97	**TONER, MEHMET**	Cellular Engineering for Metabolic Stasis	**TR**	NIGMS
98	**TRANQUILLO, ROBERT T**	Tissue-engineering Valve from Cell-Remodeled Biopolymer		NHLBI
99	**TROYK, PHILIP R.**	Intracortical Visual Prothesis		NIBIB
100	**TSUDA, AKIRA**	Particles in Developing Lung: Bioengineering Approach		NHLBI

101	**VELANDER, WILLIAM H**	cGMP Recombinant FIX for IV & Oral Hemophilia B Therapy		NHLBI
102	**VINCE, D. GEOFFREY**	High Frequency Nonlinear Acoustic Intravascular Imaging		NHLBI
103	**VO-DINH, TUAN**	Advanced Multispectral Imaging for Medical Diagnositcs	**AP**	NCI
104	**WAGGONER, ALAN S**	Long Wavelength Quantum Dot-based Probes - Cell Tracking		NIBIB
105	**WAGNER, WILLIAM R**	Cardiopulmonary Organ Engineering		NHLBI
106	**WALL, JONATHAN S**	SPECT/CT Imaging of Systemic AA-Amyloidosis in Mice		NIBIB
107	**WANG, LIHONG**	Functional Brain Imaging by Laser-Induced PAT		NINDS
108	**WEHRLI, FELIX W**	MRI of Trabecular Bone for Therapy Response Monitoring		NIAMS
109	**WEIR, RICHARD FERGUS FFRENCH**	Multifunction Prosthesis Control using Implanted Sensors	**AP**	NIBIB
110	**WERNER, JOHN S.**	Ophthalmic Imaging Using Adaptive Optics and OCT		NEI
111	**WHITE, STEPHEN H.**	Cold Neutrons For Biology And Technology	**AP**	NCRR
112	**WICKLINE, SAMUEL A**	Methods in Molecular Imaging and Targeted Therapeutics		NHLBI
113	**WILLIAMS, DAVID R**	Optics Instrumentation for Advanced Ophthalmic Imaging		NEI
114	**WITTRUP, KARL D**	Engineered Antibody EGFR Antagonist Cancer Therapeutics		NCI
115	**WOLPAW, JONATHAN R**	General Purpose Brain-Computer Interface (BCI) System		NIBIB
116	**WYATT, JOHN L**	Engineering Development of a Chronic Retinal Implant		NEI
117	**YARMUSH, MARTIN L**	Living Cell Arrays for Real Time Functional Genomics	**TR**	NIAID
118	**YU, YAN**	Robot-Assisted Platform for Intratumoral Delivery		NCI



Project Summaries

Basic

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PROJECT TITLE: Biomolecular Analysis Using Liquid Crystals

PARTNERS' NAMES AND AFFILIATIONS:

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Ronald Raines, Department of Biochemistry, University of Wisconsin-Madison
Paul Nealey, Department of Chemical and Biological Engineering, University of Wisconsin-Madison

GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI)

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 2004. A central goal of research performed under Aim 1 is to optimize the nanometer-scale topography of surfaces for detection of EGFr using liquid crystals. To date, we have used advanced lithographic and nanofabrication tools at the UW Center for Nanotechnology (CNTech) to fabricate periodic structures with lateral feature dimensions (periods from 40 nm to 80 nm) and relief dimensions (2 nm to 10 nm) at the nanoscale. We have used these surfaces in model studies to demonstrate the feasibility of detecting protein binding events with liquid crystals. In future studies to be performed under Aim 2, these nanostructured surfaces will be used to optimize the detection of binding of EGFr to antibodies immobilized on these surfaces. A second aspect of Aim 1 research is related to the development of general methods for the covalent, uniform immobilization of proteins that are both rapid and high-yielding. In collaboration with the lab of Dr. Eric Shusta (University of Wisconsin), we have

expressed high levels of a single chain antibody directed against the EGFr. We have shown that this antibody reacts selectively with the EGFr and can be expressed in a manner that will allow us to subject it to covalent and oriented immobilization on nanostructured surfaces using the expressed protein ligation (EPL) technology detailed in our proposal under Aim 1, i.e., fusion to an intein variant that enables attachment to the surface via Staudinger ligation. This immobilization methodology will allow us to have a more selective and sensitive means for capturing and assaying normal and mutant EGFr's.

Accomplishments relevant to Aim 2 of our BRP have been several-fold: (i) *Screening of antibodies.* Building from accomplishments in year 1, in which we successfully demonstrated that it is possible to combine the use of nanostructured interfaces and liquid crystals to report antibody-mediated capture of EGFr from cell lysates and membrane extracts onto surfaces, in year 2, we have carried out a systematic comparison of the capacity of several EGFr antibodies to selectively report the presence of EGFr in extracts of human A431 epidermoid carcinoma cells using immunoblotting and liquid crystal technology. We have identified several commercially available antibodies that provide consistent reporting between the two procedures (namely the H11, E235 and R111.6 monoclonal antibodies) and these are now being optimized for routine use in liquid crystal-based assays.*(ii) Feasibility of Detection of Phosphorylation Status of EGFr:* In year 2, we have demonstrated that methodologies developed in year 1 for detection EGFr can be modified to report the phosphorylation status of EGFr following EGF stimulation of intact cells. The modification involved the immobilization of a phospho-specific antibody directed towards the sequence encompassing residue pY1068. We have also demonstrated that the phosphorylation status of EGFr peptide substrates immobilized on nanostructured surfaces leads to measurable influences on the ordering of LCs. Future studies will focus on development of quantitative methods. (iii) *Detection of activity of tyrosine kinase inhibitor*. We also performed experiments in support of the feasibility of using our methodology to report the activity of known inhibitors of tyrosine kinase activity of EGFr. In these experiments, we treated cells with a tyrosine kinase inhibitor (AG1478: 5 μM, 30 min). Whereas phosphorylation of EGFr was reported in the absence of the inhibitor, addition of AG1478 resulted in no measurable phosphorylation. These results establish the feasibility of using our analytical method for evaluating the efficacy of tyrosine kinase inhibitors.

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. Towards the accomplishment of Aim 3, however, in year 2, we have made progress in several respects: First, we have now generated several EGFr mutants that are commonly found in human lung cancers and gliomas, and we have these mutants expressed in both stable cell lines or as purified proteins. Specifically, we have cell lines expressing an in-frame duplication of the EGFr tyrosine kinase domain that is commonly found in glioblastoma multiforme, and we have cells expressing the deletion EGFr mutant that encompasses a region around the ATP-binding domain (ÆELREA) that is commonly found in non-small cell lung cancer. Thus, we are now in a position to assess the capacity of the liquid crystal technology to quantify the presence of these naturally-occurring EGFr mutations in cell extracts and to ascertain their relative tyrosine kinase activity (compared to normal EGFr). Second, initial studies using conventional kinase assays reveal that the deletion (ÆELREA) EGFR mutant found in human lung cancers exhibits an altered substrate specificity, i.e., a four-fold shift in V/Km for peptide substrates such as angiotensin II (which is fundamentally new information).

ISSUES

The technical aims of the research are unchanged.

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PROJECT TITLE: High T_C Susceptometer for Magnetic Measure of Body Iron

PARTNERS' NAMES AND AFFILIATIONS:

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GRANTING NIH INSTITUTE/CENTER: National Institute of Diabetes and Digestive and Kidney Disease (NIDDK)

ABSTRACT

This Bioengineering Research Partnership combines bioengineering, basic science and clinical efforts in the design, development and clinical validation of a high-transition-temperature (high-T_C; operating at 77˚K) superconducting susceptometer for the direct, non-invasive measurement of hepatic iron stores in patients with iron overload from hereditary hemochromatosis, thalassemia major (Cooley's anemia), sickle cell disease, aplastic anemia, myelodysplasia and other disorders. Our laboratories originally proposed that storage iron (ferritin and hemosiderin) could be non-invasively assessed *in vivo* because of its paramagnetic properties. We subsequently developed low-transition-temperature (low-T_C; operating at 4˚K) *s*uperconducting *qu*antum *i*nterference *d*evice (SQUID) biosusceptometry as a clinical method for the measurement of hepatic iron stores. Non-invasive magnetic measurements of hepatic storage iron in patients with iron overload are quantitatively equivalent to biochemical determinations on tissue obtained by biopsy but the cost and complexity of the low-T_C instrument has restricted clinical adoption of the method. The low-T_C susceptometer has three elements which utilize superconductivity: (i) the SQUID, (ii) the field coils that produce a localized steady magnetic field near the liver, and (iii) the detection coils and flux transformer. During the course of our project we have constructed a series of high-T_C susceptometers for clinical studies that use (i) magnetoresistive sensors to replace the SQUID, (ii) a NdFeB (neodynium-iron-boron) permanent magnet providing a strong localized magnetic field to replace the fild coils, and (iii) detection coils and flux transformer fashioned from high-T_C "tape" operating with liquid nitrogen as the coolant. We have successfully used our third-generation high-T_C susceptometer in measurements of phantoms, normal subjects and patients with disorders of iron metabolism. The development of an affordable, readily usable instrument for the non-invasive measurement of hepatic iron will be a major advance in the diagnosis and management of patients with iron overload that should find immediate and widespread clinical use both in the U.S. and worldwide.

STATUS OF RESEARCH AND PARTNERSHIP
Highly successful and active; currently in midst of clinical studies using the high-T_C susceptometer to characterize body iron stores in patients with disorders of iron metabolism and follow effects of treatment.

ISSUES
Inter-institutional administrative arrangements and IRB coordination.

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PROJECT TITLE: Light – Directed Synthesis of DNA and other Molecules

PARTNERS' NAMES AND AFFILIATIONS:

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GRANTING NIH INSTITUTE/CENTER: National Human Genome Research Institute (NHGRI)

ABSTRACT

This University of Wisconsin Bioengineering Research Partnership is aimed at developing massively parallel light directed DNA synthesis technologies capable of rapid, inexpensive production of oligonucleotide arrays containing up to 786,000 features in as little as 4 hours. These technologies are being developed to support two main areas: **1) "Off Chip" applications**: Large oligonucleotide pools excised from the chip surface will be utilized for applications such as the production of long dsDNA (genes) and complex DNA libraries for SiRNA. The desired oligos are released from the surface then amplified in sets to form specific sub-assemblies and assembled into the final dsDNA products. Error correction methods utilizing anti-sense filter chips, and mismatch specific nucleases (Cel I) and binding proteins (MutS) have been developed to create error-free products. **2) Advanced "On Chip" applications:** Refinements and technological improvements to the original UW MAS are greatly extending the technologies' role in a number of diverse applications beyond gene expression arrays such as high-density Invader SNP assays and DNA binding site detection arrays. In addition work is currently in progress to develop ultra-high density (UHD) microarrays that will increase the number of features from 786K to a maximum of 25 million. The UHD arrays will make possible whole genome tiling or up to 25 replicate arrays on a single chip. The ability to synthesize large numbers of high quality oligonucleotides on demand, inexpensively, and with rapid turn around time, has revolutionary implications for a wide range of biological and medical research.

STATUS OF RESEARCH AND PARTNERSHIP

Below is a summary of the progress achieved in year two of the BRP grant:

Biochemistry

Development of non-MutS error filtering methods

Filter chip: We have developed and characterized the use of a 'filter chip' to enrich for the 'perfect' oligonucleotide population. The filter chip technique has reduced the mutation rate in the final product by as much as 57%. Optimization is currently underway to maximize 'filtering' ability.

Nuclease methods: We have utilized a base-mismatch nuclease (Cel I) to selectively digest duplex DNAs which contain mutations (mismatches) and have seen as much as a 45% decrease in the oligonucleotide mutation rate through the addition of this single step. Current research is focused on integrating this method with other protocols (such as 'filtering') to allow for efficient and effective enrichment of 'perfect' oligonucleotides.

Evaluation and optimization of chip synthesis parameters: We are sequencing PCR amplified chip oligos using a 454 Life Sciences Genome Sequencer 20. For the first time we can obtain a statistically significant sequence sample of the oligos produced on a chip. These experiments enable us to directly

measure the effects of chip layout, synthesis parameters and oligo release methods to maximize oligo synthesis accuracy.

Capillary multi-oligo synthesis: We have engineered a stable and scalable microfluidic synthesis cell that makes use of NPPOC chemistry and LEDs to synthesize multiple oligos in a single capillary. The cell has produced multiple mixed base oligonucleotide species within a single capillary the oligonucleotides have been show to be high quality and have been assembled into larger DNA constructs.

Bioinformatics

DNA assembly software is under development based on proven NIH DNA Works software, which divides the target genes into oligos with isothermal overlaps and is optimized for gene synthesis using "off chip" oligos.

Sequence data analysis: The direct assessment of oligos synthesis parameters using the 454 Genome Sequencer 20 generates 100,000 – 300,000 sequence reads per run. To analyze these large data sets we have developed automated scripts to sort, score and display the data on the web. This method has reduced the time required to analyze a run (>100,000 reads) from 4-5 days to 4 hours.

Chemistry

Completion of safety-catch photo-labile linker (SCPL): This linker is vital and currently being used in the examination and characterization of DNA synthesized on other surfaces (see below) where protocols utilized in silanized glass studies are not applicable

Enzymatic controlled oligo release: We developed a method of controlled oligo cleavage by first incorporating dU residue at the desired break point then treating the chip surface with a mixture of Uridine DNA Glycosylase (UDG) + Endonuclease VIII. This is a simple and inexpensive method to achieve controlled release of oligos from the chip surface.

Development high density on chip Invader SNP assay

Surface chemistry: The Invader SNP assay conditions strip oligos off the standard silanized glass substrates therefore we have developed other more durable modified glassy carbon nanocrystalline diamond substrates. The new glassy carbon substrates have demonstrated low background fluorescence and stability after multiple hybe and dehyb cycles.

Bi-Directional oligo synthesis: The Invader SNP assay requires two oligos with opposite orientation in very close proximity. We have achieved this by synthesizing on a Y-shaped linker with two different protecting groups. One group is removed and an oligo synthesized in the reverse (5'>3') direction then group is removed and the second strand synthesized in the forward (3'>5') direction. The presence of both oligos at the same location was verified by hybridization.

Engineering

The MAS system has undergone major engineering refinements in the past year, including: a new operating system, image lock algorithm refinements, overall system design improvements both in terms of individual components and subsystem interrelationships and the in-house fluid delivery system prototype has been designed and constructed and is currently being tested.

ISSUES

The majority of the effort during year two of the BRP gene synthesis program has been on improving the reliability of our gene assembly process. Problems with oligo synthesis accuracy and amplification bias have been encountered and we have designed and implemented a program of solutions. We feel these changes will result in a robust gene synthesis system.

The main challenge in our development efforts come from the need for large-scale resequecing of the synthesis products. The knowledge of the "error spectrum" is vital to improve the synthesis. We feel that access to 454 Life Sciences technologies will greatly accelerate our processes.

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PROJECT TITLE: Micromechanics of airway smooth muscle cells in culture

PARTNERS' NAMES AND AFFILIATIONS:

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GRANTING NIH INSTITUTE/CENTER: NHLBI RO1 HL65960-07

ABSTRACT

Acute narrowing of the airway lumen in asthma is driven by myosin motors that exert their mechanical effects within a cytoskeletal scaffolding that is both deformable and in a continuous state of remodeling. The mechanical properties of that scaffolding are not well defined. This BRP grant is a multi-disciplinary design-directed bioengineering project that is intended to fill that gap of knowledge. We have developed a micro-nano scale mechanical technology to measure the rheological properties of adherent living airway smooth muscle cells in culture, and the time-course of mechanical changes that occur in response to contractile stimuli or after genetic manipulation of cytoskeletal proteins.

STATUS OF RESEARCH AND PARTNERSHIP

In this BRP we have developed novel nanotechnologies to assist in the discovery of physical laws governing the abilities of the cytoskeleton to deform, contract, and remodel. These basic mechanical processes underlie a wide range of higher level phenomena in health and disease including many aspects of cancer, cardiovascular disease, malaria, and morphogenesis, but our major research emphasis focuses upon the role of these processes in airway narrowing in asthma. Investigators with backgrounds in engineering sciences, cell biology, or physics of soft condensed matter work side-by-side to pose new questions, invent new nanotechnologies, apply these technologies in novel experimental investigations, and analyze resulting data in terms of evolving mechanistic understanding of the physical properties of the living cell.

In the course of this grant we have established that cell material properties are characterized by three principal hallmarks. First, the elastic modulus of the living cell is of the order 10^2-10^4 Pa, showing that it is a very soft material indeed, and its loss tangent (or hysteresivity) is of the order 0.1, showing that it is much closer to being an elastic solid than a viscous fluid. Second, cell rheology cannot be characterized by a distinct internal time scale. Instead, relaxation times are distributed as a power law, and for that reason cell rheology is said to be scale-free. Third, material properties of the cell vary systematically with the level of tensile stress that the cell supports. Whether due to passive cell stretch or active contraction, as the tensile stress increases the storage modulus increases whereas the loss tangent decreases. Remarkably, if the cell is subjected to localized incremental loading at a constant tensile stress, then an extended linear range is observed. And if the localized loading is made even larger, then the material does not harden as might be expected, but rather softens. Taken together, these hallmarks show that cell material properties are not of any simple kind.

ISSUES

The issues that we have encountered are all highly positive. The BRP granting mechanism has allowed seamless pursuit avenues of investigation, and important collaborations, that would otherwise have been most difficult to accomplish.

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PROJECT TITLE: Development of a Bidirectional Brain Machine Interface

PARTNERS' NAMES AND AFFILIATIONS:

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

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

Having begun our second year of funding, we have made significant progress in all areas of the project, with much of the emphasis remaining on the earlier aims.

1) *To determine the effectiveness of novel decoders of neuronal signals for the control of a virtual arm.*

A paper has been submitted comparing the effectiveness of linear decoders of Cartesian hand position and joint torque. The joint torque model was implemented with and without the use of delayed position

and velocity feedback information that was intended to mimic proprioception. In addition to the linear model, we have begun to pursue kernel regression methods that explicitly allow for the introduction of nonlinearities into the prediction process. A large set of nonlinear features is computed over the neuronal activation patterns; it is over this transformed feature set that a linear function is constructed for the purposes of arm motion prediction. This nonlinear feature set increases the representational power of the full model. We have shown that polynomial kernels 1) increase the performance of arm motion predictors (over linear models), and 2) are more robust to small training set sizes.

We have also begun to make use of explicit models of muscle/spinal cord interaction in the arm motion prediction process. Our fractional power damping model takes as inputs, a low-dimensional descending muscle command signal and muscle state (length and rate of stretch), which are transformed into muscle force and joint torque signals. A training process is employed that adjusts the model's parameters, as well as the transformation from M1 cell activation to the putative descending muscle commands. Preliminary results indicate that joint torque prediction performance is comparable to our linear models. In addition, the structured model makes explicit predictions about the forces generated by the key muscle groups, and may require less training data.

Dimensionality reduction from the high-dimensional neural space to the two dimensions of the controlled task is a formidable problem that is not well solved by linear methods. We have discovered that the nonlinear *Isomap* method can preserve 2-3 times more information about the mapping from neural space than does PCA.

2) To develop a unidirectional afferent BMI to encode virtual arm state for movement guidance.

Experiments have been completed in one monkey, who demonstrated the ability to detect electrical stimulation in area 3a of the primary somatosensory cortex, and to discriminate different stimulus frequencies. A poster describing this work was presented at the 2005 SFN meeting. Experiments are underway with a second monkey that has not yet learned the task. We hope to replicate our earlier findings, and implement a new version of the task in which the monkey must respond to artificial proprioceptive illusions delivered by electrical stimulation during movement.

3) To evaluate the closed-loop performance of a unidirectional, efferent BMI with natural feedback.

In addition to the closed-loop, brain control using visual feedback described last year, we have now trained an additional monkey to perform the tracking paradigm, and have tested both using natural proprioceptive feedback in addition to vision. This was accomplished by driving the exoskeleton with the limb position prediction, and thereby moving the monkey's arm to the predicted location. Under certain conditions, the time to reach targets appeared to improve within sessions with this additional feedback. This needs further investigation.

4) *To control movements using a bidirectional BMI that incorporates sensory feedback through electrical stimulation of the somatosensory cortex.*

We have done a series of acute experiments to map the forelimb area of sensorimotor cortex in rats. This was intended to guide the implantation of chronic arrays of electrodes, which we've done now in two rats. Using these rats, we are beginning to work out the technical issues related to implantation, stimulus artifacts, and measurement of functional connectivity before attempting to implement the same methods in monkeys.

ISSUES

No issues.

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PROJECT TITLE: Image Guided Intracardiac Beating Heart Surgery

PARTNERS' NAMES AND AFFILIATIONS:

Kevin Foskett. (L.I.) U. Pennsylvania. Experimentalist – electrophysiological single-channel recording and IP3 receptor channel modeling.

Ian Parker. (L.I.) U.C. Irvine. Experimentalist – Confocal cytosolic Ca2+ imaging and modeling.

GRANTING NIH INSTITUTE/CENTER: National Institute of General Medical Sciences (NIGMS)

ABSTRACT

The overall goal of this project involves a synergistic, multi-disciplinary approach of multiscale modeling and experimental observation to elucidate the fundamental mechanisms underlying cellular calcium signaling. Cytosolic Ca2+ transients serve as a ubiquitous signaling mechanism that regulates cellular functions as diverse as secretion, contraction and proliferation. Information is encoded by spatio-temporal patterns of cytosolic Ca2+ signals at scales ranging from nanometers and microseconds to millimeters and minutes, involving 'phonemes' of Ca2+ constructed hierarchically through the activity of individual channels; multiple channels within clusters; and interactions between clusters. These levels cannot simultaneously be observed by any single experimental technique and the shorter scales are below experimental resolution. We therefore employ a dual, tightly integrated and iterative approach of data-driven mathematical modeling together with experimental measurements involving electrophysiological single-channel recording and high-resolution cellular Ca2+ imaging to elucidate how 'elementary' Ca2+ events involving individual channels and clusters are triggered and coupled to produce global cellular calcium signals. Specific aims are to: (i) characterize the gating and Ca2+ permeation properties of IP3R, and develop a predictive mathematical model to account for its complex regulation by IP3 and Ca2+; (ii) observe and model the stochastic, Ca2+-mediated functional coupling between individual channels within a cluster, and; (iii) determine the mechanisms underlying cluster-cluster interactions that allow for propagation of global signals and the powerful differential modulation of this process by Ca2+ buffers of differing kinetics. Although we focus on IP3 signaling in two experimentally-tractable systems (Xenopus oocyte, Sf9 cells) the experimental and theoretical tools we develop will be widely applicable, and the emergent principles will illuminate fundamental mechanisms of Ca2+ signaling in many cell types.

This unique integrative approach to discover the fundamental mechanisms by which intracellular Ca2+ signals are generated will fundamentally enhance our understanding of their normal

functioning and provide insights into how their disruption affects numerous diseases as varied as pancreatitis and Alzheimer's.

ISSUES
None

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PROJECT TITLE: Wear Analysis of Intervertebral Total Disc Replacements

PARTNERS:

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H. Michael Mayer, Spine Center Munich; Sergio A. Mendoza, University of Iowa

GRANTING INSTITUTE: National Institute of Arthritis, Musculoskeletal and Skin Diseases (NIAMS)

ABSTRACT

Intervertebral total disc replacement (TDR) implants potentially herald a paradigm shift in the management of degenerative disc disease. Preservation of motion may avoid adjacent-segment degeneration, a well-recognized complication of fusion. Based on encouraging experience in Europe and recently concluded IDE trials in the US, FDA approvals have been granted for two designs: Charité III (2004) and ProDisc (2005). As an alternative to fusion (~330,000/year in the U.S.), an onrush of implantations seems imminent for these two devices, and for others in the pipeline of development and regulatory review. The potential for late wear-related complications is concerning. Both currently approved devices involve metal-on-conventional-polyethylene bearings, and the TDR patient population is a decade younger than for THR/TKR. Moreover, owing to close proximity of the spinal cord and abdominal great vessels, the potential consequences of implant failure are much more dire than for THR/TKR. It is therefore crucial that the scientific community expeditiously confront the issue of TDR wear. A Bioengineering Research Partnership has been formed to provide a firm scientific basis for identifying and dealing with wear-related problems in TDR. **Aim 1** is to develop techniques for TDR wear assessment in the pre-clinical phase. This involves leveraging complementary numerical and physical techniques: sliding-distance-coupled finite element analysis (Iowa) and servo-controlled laboratory simulation (Leeds). **Aim 2** is to implement a novel approach to *in vivo* TDR radiographic wear measurement (Iowa), using high-resolution digital image analysis to assess relative three-dimensional pose position of the implant's metallic end plates. After documenting accuracy/precision, the pose image analysis technique will be used to measure wear in one of the largest/longest-ongoing European TDR series (Munich). **Aim 3** is to assess the functional biologic activity of TDR wear debris (Leeds). Morphologically realistic simulator-generated debris will be used to challenge cells in a culture preparation specifically tailored to reflect the local spinal environment, from which key metrics of the inflammatory osteolytic cascade will be assayed.

STATUS OF RESEARCH AND PARTNERSHIP

In this first year of the Partnership, work is off to a strong start in developing a technique for in *vivo wear* measurement, based on the apparent relative migration of the (radio-opaque) end plates of the

implants. An image analysis algorithm has been developed to ascertain the three-dimensional pose (position and orientation) of end plates, based upon their radiographic silhouette. Changes over time in the net separation of the end plates occurs due to loss (i.e., wear) of the intervening radiolucent polyethylene. The pose-based wear algorithm is pursuing the logic outlined in the original proposal, although we are implementing it in a more powerful software environment than originally proposed. Both manufacturers of FDA-approved devices have provided proprietary CAE files. Our algorithm computes end plate pose to an accuracy of a few hundredths of a millimeter and a few hundredths of a degree, accurate enough to estimate wear to within a few months of average clinical experience, and therefore to identify problematic wear situations. A second part of the endeavor is to automatically segment the boundaries of the implant on clinical radiographs. Here, we have been working to optimize our edge detection technique, and we have built a benchtop system with a six-degree-of-freedom micro-manipulator, in order to physically validate the accuracy of image-based pose detection.

Progress has also been good on sliding-distance-coupled finite element analysis (FEA) of wear. Here, we have been concentrating on ProDisc, the simpler of the two implants for FEA purposes, since there is only one articulating surface. Again using manufacturer CAE data, we have worked through the necessary meshing protocol, and have been obtaining well-behaved h-convergent contact solutions, for physiologic loading levels. Through Dr. Hall's liaison, we enjoy access to the current versions of ASTM and ISO draft testing protocols, and we have been computing FEA solutions and sliding-distance-coupled wear estimates through the corresponding state space.

Activities at the University of Leeds have centered around specification and development of the new five-active degree of freedom spine simulator. A number of development meetings have been undertaken with the company who successfully completed the tender process, and a conceptual design has been approved that will fulfil the original specification. The spine wear simulator has a higher specification than the range of machines currently available from commercial manufacturers, in that the important A-P shear displacement or load is included as an active element. This replicates more fully the actual spinal kinetics observed during daily activities and, therefore, provides a more realistic environment in which the wear testing can be accomplished. A dedicated researcher is now in place to further develop the wear simulation and associated activities. This will include additional protocols for assessing wear in both revision retrieved and simulator worn specimens using microCT as well as more standard gravimetric and CMM approaches.

In Munich, the annual follow-ups of Dr. Mayer's ProDisc patients are continuing as projected. A clinical research fellow has been recruited to coordinate x-ray collection for liaison with the Iowa and Leeds groups. Contiguously with the upcoming World Congress of Biomechanics, we have shipped high-resolution scanning equipment to Spine Center Munich, for on-site digitizing of x-ray images for the 50 longest-implanted of Dr. Mayer's 148 ProDisc patients. We also have established collaborative relationships with two UK spine surgeons who have large series of Charité implantations.

ISSUES

This BRP involves an international collaboration among three institutions, only one of which (the University of Iowa) is habitually a recipient of NIH grant support. Establishing the necessary administrative/fiscal structures therefore proved challenging. A second issue involves retrievals of failed implants and peri-implant tissues. To date, failures of TDRs have been infrequent and mostly have occurred for earlier-term reasons unrelated to wear-induced osteolysis. This is requiring that we aggressively network both in Europe and in the US to obtain analysis materials for Specific Aim 3.

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PROJECT TITLE: Gene Therapy for Myocardial Stunning and Infarction

PARTNERS' NAMES AND AFFILIATIONS:

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

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-α, NF-κB, 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-α, inhibiting NF-κB 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-α

knock-outs, in mice with impaired NF-κB 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 4, Dr. Prasad in our group has greatly enhanced our *in vivo* gene transfer capabilities by combining cardiac-specific promoters with the recently-identified AAV serotype 9 capsid. The resulting AAV vectors are capable of genetically reprogramming >80% the cardiomyocytes in an adult murine heart after a single IV injection. With regards to Specific Aim 4, 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). The results of these ongoing studies indicate that iNOS plays a multifaceted role in LV remodeling, with iNOS expression in different cell types contributing to LV remodeling in different regions of the heart at different times 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 Summaries prepared for previous BRP Grantee's Meetings, we identified issues relating to UVA's aging 4.7T scanner upon which a great deal of the research in this BRP depends. We are happy to report that the UVA School of Medicine recently completed the installation of a new Varian Direct Drive 4.7T MRI system to replace the previous system. In addition, the NCRR High-End Instrumentation Program recently awarded UVA $2M for a new 9.4T MR imaging/spectroscopy system. It is anticipated that these new small-animal MR scanners will support cutting-edge biomedical imaging research at UVA for many years to come.

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PROJECT TITLE: Multimodal mPET and mMRI Imaging Instrumentation

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

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 mPET 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 mPET system will interfere minimally with the mMR system, enabling high quality mPET and mMRI 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 mPET 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 mPET 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 mPET/mMRI 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 mPET studies. The 'in register' mMR images will be used to compute scatter and attenuation in the mPET 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 mPET and MRS studies of drug distributions; cardiac, CNS and tumor cell metabolism.

- Simultaneous fMRI and mPET 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

Over the past 12 months, we have made significant progress in our goal to develop an instrument capable of acquiring simultaneous in vivo microPET and microMRI images of small animal model systems. We have made advances in three specific areas:

1) development of a **MRI-compatible PET insert**: Specific milestones that have been met include optimization of the PET detector design, testing of individual detectors within a 7T magnet, study of interference between MRI and the PET detectors, design of the PET scanner, fabrication of the PET scanner, and within the last few weeks, initial testing of the PET scanner insert. Preliminary studies indicate that all the components are working, and that the MRI system is not significantly impacted by the presence of the PET insert.

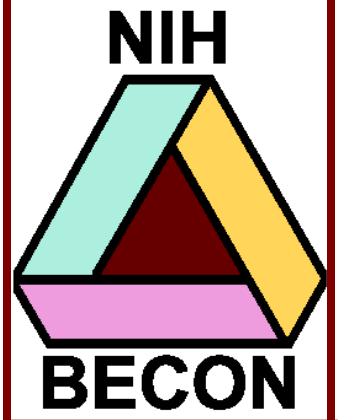
2) installation and customization of **7T/30cm MRI scanner** to accept the insert: Renovation of space in the Caltech Brain Imaging Center (CBIC) was completed in late 2005 and our 7T/30cm Bruker BioSpec scanner installed shortly after. A significant amount of time and effort has been devoted to ensuring that this space will be appropriate for implementing the in vivo imaging experiments planned in the beta-test phase of this project. All of these projects involve longitudinal studies with repeated imaging thus it is essential to setup and maintain a working environment that facilitates rapid throughput while maintaining animal health and safety. Installation and testing of the 7T/30cm Bruker Biospec went smoothly. We are now routinely acquiring high resolution *in vivo* images of several types of small animals, concentrating on a glioblastoma mouse model from the beta-test project of Dr. Andrew Raubitshek (City of Hope). We also have begun designing and building RF coils and animal holding devices for use in the joint PET/MRI instrument.

3) development of **dual mode probes** to be used in the imaging experiments: We have been synthesizing dextran (SPIO) and dextran sulfate (ADIO) coated iron oxide nanoparticles and conjugating them to copper chelators for labeling with Cu-64. The dextran sulfate coated nanoparticles are targeted for recognition by macrophages through specific macrophage cell surface receptors. Nanoparticles down to 20nm in diameter of both coating types have been synthesized, functionalized, tested on cells in culture, and conjugated to DOTA, a chelator for Cu-64.

The work outlined above has been presented at the 2005 IEEE Medical Imaging Conference, the 2006 Academy of Molecular Imaging Conference and the 2006 International Society of Magnetic Resonance in Medicine Conference.

ISSUES

We are in frequent e-mail and phone contact, and have met twice this year when Dr Jacobs traveled to UC Davis to discuss results, observe the MRI compatible PET insert in action, and plan future studies. In addition, the PIs and their group members have met at mutually attended conferences throughout the year. We have no significant issues to report.



Project Summaries

Translational

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PROJECT TITLE: Engineering Joint Scaffolds for Function/Regeneration

PARTNERS' NAMES AND AFFILIATIONS:

GRANTING NIH INSTITUTE/CENTER: National Institute on Dental and Craniofacial Research (NIDCR)

ABSTRACT

(From Grant) Tissue engineering offers considerable promise for temporomandibular (TMJ) joint reconstruction, a pressing clinical problem. To create durable engineered joint implants, the effects of scaffold material and architecture on tissue regeneration and function must be understood. To fill this vital need, we must be able to systematically study controlled scaffold architecture effects on bone regeneration, bone-cartilage regeneration, and load bearing capability. In this BRP, we will determine the effects of designed and fabricated internal architectures on bone regeneration by bone marrow stromal cells in an in vivo model of osteogenesis. We will mechanically test these architectures to determine load carrying capability. To test bone-cartilage interface regeneration in vivo, we will create a scaffold interface design seeded with bone marrow stromal cells on one half of the scaffold (bone side) and auricular chondrocytes on the other half (cartilage side), creating a bone-cartilage interface inside the scaffold. Finally, we will then engineer a prototype Conylar Ramus Unit (CRU) based on the most promising data from the bone-bone and bone-cartilage scaffold studies. The primary goals of this BRP are to: 1) Determine how two scaffold materials (hydroxyapatite (HA) and polyanhydride and four porosity variations within controlled architectures affect bone regeneration and load carrying capability. 2) Determine how scaffold interface designs using HA and polyanhydride for the bone half and polyanhydride and PGA for the cartilage half affect bone-cartilage interface regeneration 3) Test one prototype CRU scaffold that incorporates the best results from 1 and 2 in an in vivo minipig model at 3, 6 and 12 months. The prototype CRU will have designed external shape and scaffold architecture. Our first two specific aims are to apply image-based optimal design and solid free-form fabrication to create the scaffolds. The remaining four specific aims are to investigate the performance of these scaffolds mechanically and using subcutaneous models, resulting in the in vivo minipig test of a prototype CRU.

STATUS OF RESEARCH AND PARTNERSHIP

ISSUES
None

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PROJECT TITLE: Interdisciplinary Tumor Complexity Modeling

PARTNERS' NAMES AND AFFILIATIONS:

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Mike Berens, PhD (Translational Genomics Institute, Phoenix, AZ)

GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI)

ABSTRACT

Over the last three years, this partnership has developed, applied and implemented computational and physical modeling paradigms in an attempt to study the complexity of growth and brain invasion of malignant gliomas. These remain formidable tumors to treat with a mean survival of approximately on year from diagnosis. In the brain, these tumors are highly proliferative and display characteristics of angiogenesis, necrosis, and invasion. Our originally funded specific aims were:

1. **Develop and analyze an experimental 3-D in vitro model of brain tumor spheroids as they relate to growth and invasion**
2. **Develop a computational set of models of brain tumor proliferation and invasiveness**

Experimentally, we have developed the 3-D in vitro model and have analyzed the physical and material properties that affect the tumor-environment interface. Computational models reflecting such properties at the extracellular and intracellular levels continue to be generated and refined. Gene expression profile analyses have identified intracellular signaling pathways that are overexpressed in the process of invasion of tumors in normal brain. The physical and computational models are now being refined to take into account such genetic findings

STATUS OF RESEARCH AND PARTNERSHIP

The partnership's collaborative strength has been maintained by: 1- face-to-face all day meetings between the 5 research groups, carried out every 4-6 months; 2- monthly teleconferences involving members of all research groups, and 3- a web site with both public and private domains to facilitate dissemination of data between investigators (www.brptumor.org). The current status of the research is moving into a translational phase where inhibition of pathways shown to be upregulated in the process of glioma invasion in vitro are being: 1- validated in in vivo models, 2- are being employed to further refine and optimize computational and physical parameters in an effort to apply these models also for predictive

purposes with imaging data input, and 3- are being exploited to understand the role of the tumor environment on tumor growth and invasion.

ISSUES

The partnership has allowed disciplines that do not generally interact (theoretical and applied physics, engineering, mathematical biology, computational modeling, cell biology, genetics, and medical/pharmacologic sciences) to talk/discuss and come up towards solutions for the problem at hand. The challenges have been related to discussing and explaining concepts, hypotheses and experiments in a language that is understood by all – in other words, we found that a common ontology for such interdisciplinary work is still in its infancy. Furthermore, the traditional hypotheses-driven type of research favored by the biologists, the milestone-driven planned research favored by the applied scientists and engineers and the mathematical & computational-driven modeling favored by the physicist and systems biologist have required significant engagement of the teams involved to ultimately lead us to the achievement of stated aims and for the generation of future research aims.

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

PARTNERS' NAMES AND AFFILIATIONS:

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

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. 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 in vitro and rat lungs in vivo. Model proteins/peptides range from oligopeptides to proteins of biological importance (e.g., calcitonin, insulin, granulocyte-colony stimulating factor, and human growth hormone). Our research is subdivided into three major areas: i) investigation of transcellular transport mechanisms (e.g., fluid-phase, receptor-mediated and/or adsorptive transcytosis) for absorption of model drugs across the alveolar epithelial barrier, ii) elucidation of 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 of enhanced receptor-mediated transcytosis of macromolecule drugs (e.g., conjugation with transferrin in the presence of trans-Golgi disruptors). Our collaborative investigations of pulmonary protein/peptide drug absorption among several laboratories, utilizing different experimental approaches spanning cell biology to bioengineering/physiology, are providing pertinent information on advancing practical approaches to pulmonary drug delivery.

STATUS OF RESEARCH AND PARTNERSHIP

In order to characterize factor(s) in conditioned medium of cultured rat alveolar epithelial type II cell-like monolayers that enhance(s) transalveolar peptide transport, primary rat alveolar epithelial cells (AEC) cultured on Transwells for six days were dosed from the apical compartment with radiolabeled insulin or calcitonin in serum free medium (SFM), serum-containing medium (SM), conditioned medium from type I-like rat AEC (CMI), or conditioned medium from type II-like rat AEC cultures (CMII). At the end of the two hour incubation, basolateral medium was collected and the amount of peptide transported used as a biological assay for the effects of the various dosing media. For partial characterization, CMII was centrifuged in 50 kDa molecular weight cut-off Centricon tubes, and both

retentate and filtrate were used as dosing solution. Furthermore, heat denaturation and ammonium sulphate precipitation were used to determine whether the factor(s) involved maybe proteins. For mechanistic assessment, ^{3}H-mannitol was used as a marker for paracellular transport and horseradish peroxidase for fluid-phase endocytosis. Effects of temperature (4^{o}C and 37^{o}C) were also investigated. Results show that conditioned medium obtained from the apical compartment of type II-like AEC monolayers increased transalveolar transport of calcitonin and insulin by 50-80% compared to that of SFM, SM or CMI. The enhancing effect of CMII was retained in the precipitate of the ammonium sulphate treatment and in the retentate after the Centricon centrifugation. However, the enhancing effect of CMII was significantly decreased when CMII heated at 80^{o}C for 15 min was used. CMII did not affect transalveolar transport of ^{3}H-mannitol or HRP, but its effect on peptide transport was decreased when incubated at 4^{o}C rather than 37^{o}C. We conclude that conditioned medium from type II-like AEC culture contains (a) protein factor(s) which facilitate transcellular transport of peptides across cultured primary rat type II-like AEC monolayers (AEC-II).

Non-biodegradable cell penetrating peptides such as tat peptide and hepta-d-arginine exhibit potential as delivery vectors for proteins/peptides. Freshly isolated rat type II AEC were grown on permeable supports in the presence of keratinocyte growth factor to maintain type II-like cell phenotype, and were utilized for transport studies between days 5-7 in culture. Tat peptide and hepta-d-arginine were radiolabeled with ^{125}I using the chloramine-T method. Unidirectional fluxes (apical to basolateral (A-B)) were estimated from appearance of radiolabeled peptide in the basolateral compartment following apical dosing as functions of concentration and time. The amount of peptide recycling into the apical or the basolateral compartment was determined at 0, 0.25, 0.5, 1, 2, 3, 4, 24 and 48 hours following apical dosing and incubation at 37˚C for one hour. Results show that both tat and hepta-d-arginine showed a linear relationship between their apical concentrations and increases in A-B transport across AEC-II within the range of 1-20 μM. At 10 μM, A-B transport of these peptides showed linear time dependence for up to 4 hours. A one-hour pulse, followed by chase for 48 hours, showed that the peptides recycle back into the apical compartment within 4 hours, with a lesser but detectable amount transported to the basolateral compartment. We have concluded that tat peptide and hepta-d-arginine have potential to be used as vectors to deliver drugs across AEC.

ISSUES

(1) PI and Co-Investigators participate in planned meetings to develop overall strategy, encourage further collaborations, and design optimal directions for the projects. Quarterly-to-biannual scientific meetings of all personnel involved are held, at which each group presents its latest research findings.

(2) Monthly seminar series is held under the auspices of the USC Center for Drug Design/ Delivery. Recent speakers include: 10/21/05 S Sethi (SUNY Buffalo); 11/4/05 S Randell (UNC); 12/2/05 A Yu (USC); 12/16/05 E Jacobs (MCW); 1/13/06 C Waters (Tennessee); 1/20/06 K Kim (LRRI); 2/1/06 M Sunday (Duke); 2/24/06 T Blackwell (Vanderbilt); 3/3/06 U Raj (UCLA); 3/17/06 A McDonough (USC); 3/31/06 G Toews (Michigan); 4/7/06 S Bellusci (USC); 4/28/06 J Peti-Peterdi (USC); 5/26/06 S Hamm-Alvarez (USC); 6/9/06 B Driscoll (USC); 6/16/06 S Idell (Texas); and, J Belperio (UCLA).

(3) We are studying mechanisms of absorption of peptide/protein drugs across the alveolar epithelial barrier via various transcytotic processes and exploring the effects on absorption of transport enhancers (e.g., components of bronchoalveolar lining fluid or ethanol).

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PROJECT TITLE: Microchip devices to assay quantal exocytosis

PARTNERS' NAMES AND AFFILITATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke (NINDS)

ABSTRACT

Peptide hormones and neurotransmitter are stored in membrane-bound vesicles within endocrine cells and neurons. Upon stimulation, a rise in intracellular Ca^{2+} 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 outlined below.

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

Aim 1. Target individual cells to electrochemical microelectrodes on microfabricated devices.
We are continuing to develop 2 approaches to transport cells to specific docking sites on microchips. In one approach we are etching microfluidic channels in silicon wafers. "Cell-docking sites" are patterned on the chip near fluid channels that are too narrow (1 – 2 μm wide), to allow cells to pass. Over the past year we have successfully demonstrated targeting of cells to the docking sites and have added platinum electrodes to serve as electrochemical electrodes for measuring quantal exocytosis. A further development has been integration of the silicon chip onto a printed-circuit board containing amplifiers for electrochemical measurements. We have some initial electrochemical measurements of cell secretion, but further tests are necessary to establish conditions for reliable measurement of quantal exocytosis.

Our second approach has been to trap cells in shallow microfluidic channels formed by molding the flexible polymer poly(dimethylsiloxane) (PDMS) over multi-layer photoresist. Our challenge over the last year has been to align the trapping sites on the PDMS slab with patterned electrodes on the glass substrate to enable electrochemical recording from trapped cells. Here we used wet etching to pattern an Indium-Tin-Oxide (ITO) film on a glass substrate (Anal Chem. 78: 2521-2525, 2006). The PDMS slab

containing the microfluidic channels is aligned to the ITO working electrodes using a custom-built aligner working under a high-power (200x) microscope. Our preliminary results indicate that we can resolve amperometric spikes, the signature of quantal exocytosis, with this configuration, thus the alignment of the working electrodes with the cells must be within several microns.

Aim 2. Develop approaches to stimulate exocytosis from cells on microdevices. Over the past year we concluded a set of experiments aimed at determining the rate that we can exchange the solution surrounding a cell as it flows through a microfluidic junction. The aim of these experiments is to test the feasibility of stimulating exocytosis with rapid solution exchange. We labeled the surface membrane of cells using the fluorescent styryl dyes FM1-43 and FM2-10. These dyes fluoresce only when inserted into membranes. Labeled cells flow through a PDMS microfluidic channel where they encounter a junction with dye-free solution. The decay in cell fluorescence is then measured as an indication of the loss of the FM dye from the cell membrane. The fluorescence decay thus reflects a convolution of the rate of solution exchange at the surface membrane and the rate that dye departitions out of the surface membrane. Co-I Howard Hu conducted a set of numerical calculations to estimate the rate that the FM dye is washed out of the solution surrounding the cell thus enabling estimation of the dye departitioning rate. The results of these simulations indicate that the solution surrounding the cell can be ~90% exchanged in ~ 20 ms. Combining these numerical simulations with experimental measurements of fluorescence decay indicates that the FM1-43 dye departitions from the cell membrane with a time constant of ~70 ms. This departitioning rate is of interest because FM dyes have been used to estimate the minimum time that a vesicle remains in contact with the surface membrane during "kiss-and-run" exocytosis. Thus our measurement of fast FM1-43 departitioning supports models of rapid kiss and run exocytosis in hippocampal synapses.

Aim 3. Integrate carbon-based electrochemical electrode materials into microdevices to increase sensitivity and performance. We are using self-assembly of carbon-based nanostructures in a silica matrix to construct electrodes. Using this approach we have produced electrodes with high conductivity, excellent stability, and a large electrochemical working window. Our second approach has concentrated on deposition of diamond-like carbon (DLC) microelectrodes by DC magnetron sputtering. By using the lift-off technique and nitrogen doping of the DLC film, we can fabricate conductive DLC microelectrodes in one step, a fast process compatible for batch production and at a much lower cost than the use of noble metals. Tests of these materials show a large electrochemical working window and we have successfully recorded quantal exocytosis from chromaffin cells using these electrodes. In addition, our work demonstrating the usefulness of Indium-Tin-Oxide as a transparent electrochemical electrode material for measurement of quantal exocytosis was recently published (Anal Chem. 78: 2521-2525, 2006).

Aim 4. Develop electronic instrumentation to allow simultaneous recording of many channels of electrochemical or electrophysiological data. We are developing CMOS devices capable of measuring transient amperometric oxidation currents at the surface of an electrode with submillisecond time resolution and picoampere current resolution. The amplifier is a regulated cascode stage in which a high-gain amplifier maintains the sensor voltage through a negative feedback loop. The amplifier array uses a new shared amplifier structure in which all of the amplifiers in a given row share a common half circuit permitting us to use fewer transistors per amplifier. Test chips were fabricated in 0.5µm, 5-V CMOS process through MOSIS. Each amplifier occupied a layout area of 35µm x 15µm and contained eight transistors and a 50-fF integrating capacitor. The charge resolution is approximately 0.45pA and the maximum charge storage capacity is 1.26×10^6 electrons.

ISSUES

We have no significant issues to report.

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PROJECT TITLE: MEMS Sensors for Arrhythmia Detection and Intervention

PARTNERS' NAMES AND AFFILIATIONS.

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

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 sonicMEMS group at Cornell University. 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.

Analog voltage measurements are processed and converted to digital signals by a separate off-board circuit. Each signal-processing PCB is capable of handling ten channels, which accounts for all signals measured from the electrodes on each probe. The circuit incorporates a 60Hz notch filter by combining a low-pass and high-pass filter to remove outside noise, and an amplifier to boost signal. After filtering and amplification, the signals are recorded by a multi-channel data acquisition system.

Analysis of the signals recorded during ventricular fibrillation has been aided by parallel computer simulations in which fibrillation is produced in an anatomically realistic recreation of the electrical activity of the canine ventricle. The temporal signal recorded at each measurement point, both from the computer model and from the experimental preparations, is Fourier transformed with a real to complex FFT, all values for negative frequencies are zeroed and a reverse FFT is used to transform back to the time domain. After conducting the procedure on a grid of spatial points, the resulting complex waveform can be used to find phase singularities, which are the pivot points about which spiral waves of excitation circulate. Such waves are believed to be the underlying cause of the rapid and fragmented wave propagation that occurs during fibrillation.

To find the spiral cores (or filaments) the circulation is calculated around each spatial point. A spiral core (or filament) is located at the position where the circulation $\Gamma = \pm 2\pi$. However, calculating the phase $\phi(x,y,z;t) = \text{atan}(\xi_{im}/\xi_{re})$ at each spatial point can be very time consuming. If only spiral cores or filaments need to be found, time can be saved by evaluating first the signs of the real and imaginary parts. The spiral cores are then tracked during fibrillation to determine spiral wave lifetime and the degree to which intact spirals meander. Currently, experiments are being conducted to test whether flattening of the restitution relation for action potential duration prevents spiral wave formation or, in the presence of existing fibrillation, prevents spiral wave break-up and terminates fibrillation.

ISSUES

Additional computer simulations and experiments are needed to determine how many recording sites are needed to accurately characterize wave propagation in the entire fibrillating heart. In addition, it would be useful to develop powered devices, to reduce the tethering effects of the wires and, ultimately, to produce devices that could be implanted chronically and recorded from non-invasively.

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PROJECT TITLE: Micro-electric Impedance Spectroscopy (microEIS) of Hair Cells

PARTNERS' NAMES AND AFFILIATIONS:

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GRANTING NIH INSTITUTE/CENTER: National Institute on Deafness and Other Communication Disorders (NIDCD R01 DC04928)

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. MicroEI 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. Perhaps more importantly, a subset of the technology is finding practical application in cost-effective point-of-care flow-cytometry systems. 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 first year of funding following a competitive renewal (5th year overall) . 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 microEI 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 and flex systems. 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 f_n~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 nonlinear 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 similar technology is under development for automated hematology analysis by one of the BRP partners. The company E.I. Spectra (H.E. Ayliffe; Seattle WA) was founded, in par, 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 taking initial steps toward 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: Cellular Engineering for Metabolic Stasis and Biopreservation

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GRANTING NIH INSTITUTE/CENTER: NIBIB

ABSTRACT

With the advancements being made in tissue engineering, cell transplantation, stem cell biology, and gene therapy, the clinical demand for effective long-term storage methods for cells and tissues will continue to increase. We propose to develop novel methods to biostabilization of mammalian cells for long-term preservation in a **desiccated state** at ambient temperature. 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. 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, storage, and rehydration conditions (i.e., physicochemical, biochemical, and metabolic) can be used to achieve desiccation tolerance in mammalian cells and tissues. This project is inspired by nature and it uses engineering approaches to translate nature's solution to long-term storage or "suspended animation" for mammalian systems.

STATUS OF RESEARCH AND PARTNERSHIP

We utilized several cell lines, which are known to express endogenous ATP^{4-} sensitive $P2X_7$ receptor channel, to load the desiccation stabilizing sugar trehalose into the cytoplasm of these cells. This channel can be precisely toggled between a close and open state by controlling the ATP content in the suspending medium. We have completed a detailed set of studies characterizing the molecular transport of trehalose through the $P2X_7$ receptor channel (Elliott et al., *Cryobiology* 52: 114, 2006). Cells that were loaded with trehalose were then dried to various final moisture contents under controlled conditions. Cells dried in the presence of intracellular trehalose showed superior survival at all moisture levels investigated compared to cells without trehalose. The desiccation tolerance was significant for moistures levels down to 8 to 10%. Further drying resulted in compromised integrity of the cells. These studies establish the baseline for the following metabolic engineering of cells prior to desiccation to further impart desiccation tolerance to mammalian cells.

With respect to pre-conditioning metabolic studies, we developed a new method to control the metabolic state of cells prior to desiccation. 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.

We also identified using DNA microarray technology that cyclophilin D gene expression is down regulated 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. When mammalian mitochondria are exposed to high calcium concentrations in the presence of the co-activator P_i, 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 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 and will be further investigated over the next two years.

We have also initiated our studies to investigate the mechanism of cell death and the role of apoptosis in desiccation. Multiple pathways exist for the release of the pro-apoptotic molecule cytochrome-c from the intermembrane space of mitochondria, including the MPTP and outer membrane permeabilization mediated by BAX and BAK. Regardless of the mechanism of cytochrome *c* release, the effect of elevating cytochrome c in the cytoplasm is to polymerize with APAF-1 in the presence of $_d$ATP/ATP to form the apoptosome, which then activates initiator caspases like caspase 9 and executioner caspases like caspase 3. We have recently observed that cytochrome *c* does not promote caspase activation in organelle-free extracts from *Artemia* embryos. While the proximal reason for this lack of effect of cytochrome *c* is unclear at present, the phenomenon is in marked contrast to that seen in mammalian cells using the same techniques. Thus, the additional safeguard to avoid activation of the apoptotic cascade in *Artemia* embryos suggest that biochemical intervention at the point of apoptosome formation could be a powerful mechanism to improve mammalian cell survival after desiccation storage. Thus in the coming year, we will plan to test this hypothesis by applying inhibitors of caspase 9 and/or using RNAi to depress the expression of APAF-1 – both approaches would be predicted to depress apoptosis.

To test whether trehalose needs to be present in mammalian mitochondrial matrix for a high degree of desiccation tolerance, we introduced trehalose into isolated hepatocyte mitochondrial matrix by reversibly permeabilizing mitochondrial inner membrane using MPTP. The concentration of intra-matrix trehalose reached 0.29 mmol/mg protein (~190 mM) in 5 minutes. The mitochondria with and without permeabilization were desiccated in a buffer containing 0.25 M trehalose by diffusive drying. After re-hydration, the inner mitochondrial membrane integrity was assessed by JC-1 measurement of mitochondrial membrane potential. 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 (Liu X-H et al., *Biochim. Biophys. Acta* 1717: 21, 2005).

ISSUES

We have not encountered any serious limitations or issues with the project. We have regular (at least quarterly) face-to-face meetings, conference calls, and various other means of communication between the members of the partnership. Dr. Steve Hand and one of his postdoctoral fellows Michael Menze have visiting scientist appointments at the MGH and are planning to spend several weeks at MGH during the summer to perform experiments that require close working relationship between the members of the partnership.

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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 (NIBIB)

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 research 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 ^{125}I (27.5 keV), ^{99m}Tc (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 reconstructed 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 sympathetic 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 combines 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

1. Development of a pinhole SPECT system for small animal imaging: We completed the hardware and instrumentation development phase of the project (Specific Aims 1-3). A major effort, headed by our partner, Gamma Medica-Ideas, Inc., (Northridge CA) (GMI), has been devoted to the development of two state-of-the-art 80×80 cadmium zinc telluride (CZT) detectors for radionuclide imaging. Our partner and mechanical engineering subcontractor, James Carver of Jamco Engineering (Cottage Grove, OR) has developed lead detector housings and pinhole collimators and they have been delivered to UCSF and have been integrated with the

CZT detector arrays from GMI. Two complete CZT detectors have been configured and tested, and have demonstrated excellent energy resolution at 22 keV (^{109}Cd), 60 keV (^{241}Am), and 122 keV (^{57}Co) as surrogates for ^{125}I, ^{201}Tl, ^{99m}Tc. We also have received and are evaluating a third CZT detector with 1.5 mm pixel pitch in a 128×128 array in the same 20×20 cm^2 format as the 80×80 detectors. This high resolution third detector is under initial test and it will be integrated with the imaging system over the next few weeks.

2. Development of dual-modality imaging system: The second specific aim targeted the integration of a SPECT/CT system for small animal imaging. A significant challenge was developing a system with sufficient mechanical accuracy of rotational positioning to achieve the 50 m spatial resolution needed for microCT, while supporting the significant weight of the gamma cameras lead housing. Our partner and mechanical engineering subcontractor, James Carver of Jamco Engineering (Cottage Grove, OR) has developed the scanner gantry and all the required mechanical components to operate the microSPECT microCT imaging subsystems. During the last six months Jamco Engineering focused in the development of the mouse bed with high precision linear motion to add the capability to do helical scanning. The mouse bed will be heated using a carbon-fiber resistive pad with feed-back control to maintain physiology mouse body temperature. The mechanical components have been tested using phantoms and pilot studies with animal models.

3. Develop cone-beam tomographic reconstruction algorithms for microCT and microSPECT: We have completed the development and evaluation of filtered backprojection reconstruction algorithms for microCT, and of iterative algorithms for reconstruction of the microSPECT data including the use of CT-derived attenuation maps for correcting the microSPECT data for photon attenuation. We have implemented algorithms to acquire with helical scans and reconstruct high resolution microCT and microSPECT scans. We numerically simulated and reconstruct multipinhole microSPECT data and microSPECT data with large diameter pinholes to evaluate different designs and strategies for substantially improving detection efficiency while maintaining spatial resolution, and with the goal of significantly shortening the acquisition time of the microSPECT system, or decreasing the amount of injected activity and thereby reduce radiation dose delivered to the animal.

4. Radionuclide quantification techniques: We 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. We are evaluating techniques that use CT-derived volumes-of-interest (VOIs) to quantify radionuclide uptake from the microSPECT data, to determine if this technique offers improved accuracy and precision in comparison to quantification techniques that define the VOIs on the radionuclide data alone. Finally, we are evaluating an algorithm to correct cross-talk errors in the detector for dual-isotope studies.

5. Small Animal Imaging Studies: Our small animals studies have being changed to take advantage of a new partnership between our group and Hermes Biosciences Inc. (South San Francisco, CA). We performed preliminary experiments on a mouse model of breast cancer treated using a liposome drug delivery system containing chemotherapeutic drugs. Our initial studies imaged ^{111}In-labeled liposomes to measure biodistribution needed to assess therapeutic efficacy. At the present we simulating and designing multipinhole collimators to reduce the amount of injected drug from 300 mCi to 20 mCi (to reduce radiobiological effects) and to reduce scanning time from approximately 8 to 1 hours so that the animal can be maintained under anesthesia during the SPECT/CT scan. We also have initiated a collaboration with Dr. Katherine Matthay in the UCSF Department of Pediatrics, and with Dr. William Weiss in the UCSF Department of Neurology to use SPECT/CT to assess biodistribution and to perform Dosimetry of therapeutic ^{131}I-MIBG in a transgenic mouse model of neuroblastoma.

ISSUES

Small animal imaging has become an important tool in research of mammalian biology and human disease. The small animal SPECT/CT system being developed within this BRP is designed for high resolution imaging of structure and function with the goal of improving both the visual quality and the quantitative accuracy of radionuclide imaging systems in comparison to conventional small animal imaging techniques. It is not surprising therefore that companies (including our partner GMI have developed small animal SPECT and SPECT/CT systems that now have made these available commercially. It therefore has been a challenge to manage our partnership within the context of this rapidly evolving field, but we nevertheless are making good progress through the efforts of the personnel at UCSF and of our partners in this BRP.

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PROJECT TITLE: Living Cell Arrays for Real-Time Functional Genomics

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

ABSTRACT

New tools are revolutionizing biomedical research, enabling an exponential growth in the acquisition of data regarding genes, proteins, and their structure and function in normal and diseased states. Particularly notable among these advances is the ability to monitor profiles of genes on a large scale. Despite the success that expression profiling has enjoyed, it is often difficult to correlate the trends and relationships observed in normal and abnormal states to the phenotype resulting from the gene expression profile. Pathological states that involve inflammatory responses are usually associated with metabolic derangements as well, in which case gene expression profiling does not fully explain the complex molecular mechanisms involved. Thus, in order to develop a comprehensive understanding of the interaction between metabolic states and inflammatory responses, it is essential to understand both the gene expression events as well as the cytoplasmic events, which control changes in metabolites. The proposed Bioengineering Research Partnership seeks to develop a new functional genomics approach for studying gene expression which relies on intact cells for the simultaneous temporal expression profiling of multiple genes using aequorin-type fluorescent proteins (AFP) in a massively parallel, high throughput format.

In the proposed studies, this system, which we call the "Living Cell Array" (LCA), will be used to investigate the regulation of inflammatory signals in the context of steatohepatitis. More specifically, we will characterize the effect of intracellular lipid accumulation in cultured liver cells on the regulation of inflammatory cytokine (for example tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6) signal transduction and its impact on the cellular phenotype (for example release of chemokines that attract inflammatory cells to the tissue after injury). Applying the concepts of the LCA to this problem will help test and validate this tool for studies on a variety of other inflammatory disorders, and on chronic complex disease, in general. Our specific aims are:

Specific Aim 1: To generate and characterize a panel of reporter cell lines that monitors the major events in the IL-6, TNF-α, and IL-1β signaling cascades.

Specific Aim 2: To design a microfluidic system to dynamically control the input stimulus as well as the fluorescent response of an array of H35 reporter cell lines.

Specific Aim 3: To characterize the dynamics of TNF-α, IL-1β and IL-6 signal propagation in H35 cells and the impact of steatosis on this response.

This Bioengineering Research Partnership combines the expertise of several investigators in different disciplines: (1) state-of-the-art molecular biology and genetic engineering techniques; (2) microfabrication techniques and transport phenomena; (3) hepatocellular physiology in inflammatory states; and (4) advanced microimaging methods. By assembling this group of investigators and their laboratories, we will develop the first living cell array that can monitor signal transduction in real-time. As a test case, the array will be used to

elucidate the effect of steatosis on inflammatory signal transduction in the context of mechanisms that lead to hepatic fibrosis.

This project has the potential to have a significant impact in a number of ways. In the short-term, the proposed studies will (1) elucidate the effect of steatosis on signal transduction in hepatoma cells; (2) provide a rational basis for altering signal transduction processes in liver that are relevant to steatohepatitis and fibrosis; (3) provide a tool that can be used to investigate other inflammatory conditions of liver. The long-term outcomes of this project are scientific and technology bases to develop living cell arrays that can be used to study other inflammatory liver diseases as well as diseases in other organ systems.

STATUS OF RESEARCH AND PARTNERSHIP

Our partnership has been strong and productive. We have presented the work six times at scientific symposia, submitted two manuscripts related to the project, and have one in preparation.

During the initial period of funding, we have dedicated significant effort to characterization and validation of the stably transfected reporter cell lines reported in the preliminary data, as well as the generation of new stably transfected reporter lines. We have also made significant progress in the development of advanced microfluidic systems that provide improved control over the administration of combinatorial stimuli. Finally, we have generated data that represent a fusion of these efforts and that will serve as a baseline control for our current and upcoming studies on steatotic liver cells.

We have focused significant effort on characterization and quantitative evaluation of six stably transfected cell lines (NF-kB, AP-1, HSE, GRE, ISRE, and STAT-3), which will serve as a starting point for our characterization of cell signaling perturbations induced by steatosis. Recently, we have further expanded our reporter plasmid library with the addition of STAT-1, STAT-4, STAT-5, and CEBP, which we are currently transfecting into the H35 hepatocyte-like cell line. In addition, we have eight more plasmid constructs under development and expect to have a library of approximately 20 to 25 reporters by the end of 2006.

The power of the LCA approach is derived from the ability to monitor a *dynamic* cellular response, in real-time, and in a high throughput manner. To demonstrate this, we conducted an experiment in which six cell lines were each stimulated with six classical inducers and the fluorescent response quantified over 24 hours. These results represented a proof-of-principle for the LCA concept and demonstrated acquisition of over 5,000 single-time-point measurements, on six reporters, in a 24-hour period. It would require weeks to months to acquire similar data using traditional Western blot or electromobility shift assay methods.

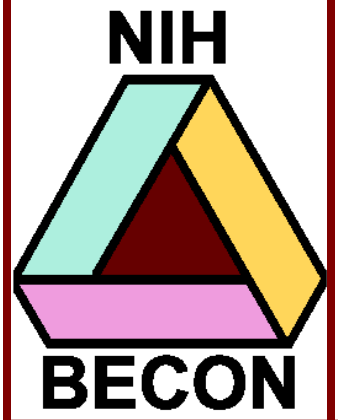
In addition to these results, which were generated using lean H35 cells, we have begun to optimize the medium requirement to obtain steatotic (fatty) H35 cells. One issue we have faced in doing this is that the H35 cells do not become as fatty (microsteatosis) as primary hepatocytes (macrosteatosis) upon exposure to our typical steatosis-inducing medium. We are currently working on optimizing this medium to obtain H35 cells with acceptable levels of steatosis.

In addition to the characterization of the cell lines, we have also made progress in the design of improved microfluidic systems for stimulus control. The microfluidic system used to collect the data described above consists of an 8x8 array of fluidic cell culture channels that can be isolated by the application of negative pressure to a series of integrated valves. Using this technology, we are able to seed multiple reporter cell lines on the same chip, with no cross-contamination between cell culture areas. Likewise, we are able to direct stimuli to specific channels, also with no cross-talk.

In addition to the fluidics described above, we have also developed a new microfluidic stimulus control system, termed Flow-Encoded Switching that enables us to exert precise temporal control over the stimulus reaching the clones in the cell culture channel. This system relies on manipulation of the differential pressure at two inputs to control stimulation time, recovery time, and frequency. Using this technique, we now have the ability to administer transient cytokine stress, vary the time of recovery from cytokine stress, and administer pulsatile cytokine stress, all methods that we expect to increase the flexibility of our experiments, as well as the efficiency at which they can be performed.

ISSUES

Outside of the normal experimental challenges, there have not been any problems with the administrative or technical aspects of the partnership.



Project Summaries

Applied

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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 (NINDS)

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, and The 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 fifth 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 (This includes expanding the algorithms to new seizure precursors, specifically high frequency epileptic oscillations, developing methods using machine learning to train and run 2nd generation implantable epilepsy devices that require minimal training to individuals, developing other streamlined methods for online seizure prediction algorithms for online accumulated energy calculation, and developing a method for fusing multiple quantitative features for seizure detection/ prediction within a 10 minute prediction horizon), (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. We have seen progress 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, double blind, controlled clinical trial of a responsive, implanted brain stimulation device to predict/detect seizures and stimulate to prevent seizures. 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 three 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) Examining changes in excitability in multiple brain regions in epileptic rats, and (3) Extending the long term chronic recordings to awake, unanesthetized epileptic rats.

Circuit and cellular biophysical mechanisms during generation of the preictal cascade. We have continued 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 in the chronic epileptic hippocampus, the dentate gyrus acts as a filter, determined primarily by feedforward and feedback GABAergic inhibition, regulating entry of information and seizure activity. 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.

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 either pilocarpine-induced or perforant pathway stimulation-induced status epilepticus and are identifying patterns of EEG activity that are correlated with impending seizures. We have also identified some rats with hyper-periodic seizure clusters and are examining features of these clusters. We have begun preliminary open and closed loop stimulation of hippocampus to try to alter the seizure pattern.

ISSUES

Developing animal models of either acute or chronic seizures that appropriately mimic the human disorder 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. Many of these models have extensive brain damage. In addition, it is clear that long term monitoring of epileptic animals is needed to analyze how the epilepsy develops after an inciting stimulus and the pattern of seizure generation occurring over time. The lack of a non-tethered multichannel high bandwidth system for chronic recording is a major impediment to these types of experiments. Our data from animal models also suggest that such a chronic monitoring system would likely be very valuable for human patients with epilepsy. Despite these caveats, the seizure prediction activities are proceeding rapidly in both humans and the animal models.

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PROJECT TITLE: Anti-Inflammatory Coatings for Biomaterials

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

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, and Synthetic Blood, Inc), 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: We have defined the mechanism by which titanates acts as anti-oxidants. We have discovered that titanate perovskites and niobium-doped titinate have better anti-oxidant properties than titanium dioxide Project 2: Micropatterned titanium implants have been implanted in rats for 4 weeks. The implants have been excised and are undergoing histological analysis. Project 3: Perovskite and Niobium-doped titanate-coated silicone implants were implanted in rats. We are processing the retrieved implants by histology. 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 have established a relationship with Synthetic Blood, Inc. Scientists at this company had pioneered oxygen sensors and have developed an implantable glucose sensor. We expect to submit a Phase 2 STTR to further develop this BRP-based technology.

ISSUES

None.

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PROJECT TITLE: Image and Model Based Analysis of Lung Disease

PARTNERS' NAMES AND AFFILIATIONS:
Johns Hopkins University, Marquette University, University of Washington, Mayo Clinic/Foundation, University of Texas, University of Auckland, Siemens Medical.

GRANTING NIH INSTITUTE/CENTER: Primary: National Heart Lung and Blood Institute (NHLBI); Secondary: National Cancer Institute (NCI)

ABSTRACT
With the emergence of therapeutic interventions for many common lung diseases, there comes a critical need for sensitive and objective measures of regional lung status both for detection of disease and outcomes analysis. In the last 5 years it has been increasingly recognized that new approaches to diagnosis and therapy of lung disease offer substantial benefits to selected populations. These approaches include, but are not limited to, lung volume reduction surgery, percutaneous and transbronchial approaches for diagnosis and interventions; early lung cancer detection and evaluation; and recent advances in the pathological classification and treatment of the interstitial lung diseases. In all of these instances, X-ray CT remains the imaging modality of choice for comprehensively evaluating the lung, due in part to significant advances made in both temporal and spatial resolution, as anticipated by our original BRP submission for which we are now seeking renewal. Multidetector-Row CT (MDCT) scanners are now capable of subsecond (375msec and faster) data acquisition speeds allowing for the imaging of not only anatomy, but also ventilation and perfusion, providing structure-to-function correlations. With the rapid widening of the cone beam on these scanners through the addition of more detector rows, true volumetric imaging is on the horizon. Critical to the field of pulmonary medicine is a better description and understanding of the human lung and its response to injury, based not upon global measures but upon quantifiable regional features. This approach recognizes the complex regional control of lung ventilation and perfusion as a fingerprint of lung function in health or dysfunction in disease quantified for the first time by our original BRP with a level of detail only achievable in humans by dynamic CT technologies. A major limitation in the development of new therapies for lung diseases is that global outcome measures do not adequately capture lung complexity and are minimally altered by significant local disease, resulting in the need to study large numbers of subjects over long time periods. CT measures are increasingly recognized as very sensitive indicators of subclinical disease, based upon regional measures, and appear to much better describe these complex lung processes. Small changes are easily detected and quantified, particularly using computer aids, resulting in a more rapid, and more objective assessment of therapeutic outcomes. However, at the same time, the increasing propensity of public policy to mandate increases on the limits of the use of ionizing radiation is promising to restrict the full deployment of the newly emerging quantitative tools CT has to offer for assessing detailed lung structure-function relationships in the early detection of pathology, the temporal evaluation of disease progression, and in the evaluation of success of therapeutic interventions.
As we continue into our fifth year of our BRP and seek its renewal, we bring together a multi-disciplinary team of investigators (a "bioengineering partnership"), some new and many from the beginning of our partnership to further advance lung imaging and to build an atlas/model (including anatomic measures as well as measures of regional ventilation and perfusion) of the normal male and female adult human lung such that the individual can be compared to the atlas / models for early detection of disease and sensitive evaluation of disease progression or intervention outcomes. All of these data (within HIPAA guidelines) will be made publicly available through our MIFAR (medical image file archive) open source software project such that this normal atlas will serve as the baseline for other imaging explorations of the lung. The CT methods we are developing

will, and are, serving as a gold standard against which other complimentary imaging modalities can be calibrated and validated.

STATUS OF RESEARCH AND PARTNERSHIP

We have made strong advances in all aspects of our proposed research, as testified to by both our publications, the increasing strength of our collaborations, successful spin-off grants and SBIR applications, strengthened partnership with our newly acquired partnership with Siemens Medical, and successful patent applications. Milestones in successful proliferation of our technologies include:

A state-of-the-art imaging research facility (Iowa Comprehensive Lung Imaging Center or I-Clic) which includes multi-detector row CT (MDCT), micro CT, color bronchoscopy, and microscopy to support our partnership has been completed at the University of Iowa and we have forged a partnership with Siemens which will maintain the MDCT facility at pre-beta level state of the art for a minimum of the next 6 years. We have completed design and fabrication of what may be the world's largest vibrating microtome (LIMA) with associated computer controlled microscope hardware for the 3D pathology evaluation of *ex-vivo* specimens .

We are creating a unique, open source database environment which will: 1) allow storage of arbitrary types of data on multiple computers; called the Medical Image File Archive system (MIFAR). The heart of the system is a relational database that coordinates the activities of multiple small server programs spread across a network and with a browser interface. http://dpi.radiology.uiowa.edu/mifar/index.php

We added capability of doing Multiple Inert Gas Elimination Technique studies and fluorescent microsphere maps of flow and ventilation in the lung through a supplement to the BRP with University of Washington (Dr Mike Hlastala, PI)

BRP techniques were involved in several additional grants being awarded (Dr Brett Simon D.O.D. funding to study exogenous surfactant in acute lung injury and project leader on an NIH Acute Lung Injury SCCOR; Drs. Wang, Hoffman and McLennan NIH funding to use an extension of our lung imaging methodologies in micro-CT imaging of mice; J Garbor, EA Hoffman, J Reinhardt, G. McLennan, and M Sonka, SBIR R43-HL075953, "Tissue and airway evaluation of emphysema interventions."

Aventis Pharmaceuticals has purchased a commercial micro CT scanner from Skyscan for placement within the I-Clic. This scanner will serve to help us understand the 3D *ex vivo* anatomy of tissues imaged *in vivo* via our CT protocols. In addition the scanner will help us move some of our imaging methodologies towards the study of mice with genetically based lung pathologies.

Our paper on Xenon CT for use in assessing regional ventilation [1] and our paper on a method for assessing MDCT slice geometry were awarded the Association of University Radiologist's Herbert M. Stauffer, Outstanding Basic Science Papers for the years 2002 and 2003 respectively.

While we do not believe that numbers of papers published are the best measure of success in the unique category of funding established in the creation of the Bioengineering Research partnership, we have, as a group published XX papers and XX abstracts and have XX works submitted or in press. An overview paper of our work was featured recently in Academic Radiology [3].

We are currently utilizing newly developed methods for lung, lobe airway and vascular segmentation, automatic anatomic labeling, image matching, blood flow and ventilation analysis to populate the atlas of the normal human lung.

Our BRP derived image analysis software was used in the recently completed National Emphysema Treatment Trial (NETT), and the objective computer-based measures were able to provide highly significant predictions of subject specific probability of positive surgical outcomes and a subgroup with significantly reduced mortality relative to the non-surgical group.

ISSUES

Our original partnership began with Picker. As Picker became Marconni and Marconni became Philips, the relationship with the company changed in ways which caused us to re-assess our industrial partnership. This re-assessment lead us to the establishment of a new relationship with Siemens Medical which has now provided the partnership with access to beta-level state-of-the-art CT scanning hardware for at least the next 6 years and a strong interactive relationship with the Siemens CT development team.

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

PARTNERS:
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GRANTING INSTITUTE:
National Cancer Institute (NCI), National Institute of Biomedical Imaging and Bioengineering (NIBIB)

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 advances in several aspects of the project. First, we have improved the efficiency of both excitation laser delivery and fluorescence signal collection by employing a bifurcated fiberscope for the *in-vivo* SL-based imaging system. This bifurcated fiberscope design (Fig. 1) with a common distal end for the excitation fiber bundle and collection fiber bundle replaces the previous configuration with two separate fiber bundles for excitation and collection, respectively.

The new fiberscope consists of an excitation fiber bundle and an imaging fiber bundle with 10,000 fibers. This configuration offers several advantages over the previous design: First, the relative position of the excitation fiber bundle to the collection fiber bundle is fixed and reproducible from measurement to measurement, thus significantly improving the reproducibility of the measurements. Secondly, by combining the distal end of the excitation and collection bundle, only one biopsy channel is required for the LIF measurements, making it simpler for the physician to implement clinically. In addition, the combination of the distal ends also enables better overlap between the area that is excited by the laser and the area that is being imaged by the collection fiber bundle. Finally, the transmission efficiency of the new excitation fiber bundle is much better than that of the previous excitation fiber bundle. As a result, more energy can be delivered to the target tissue in the esophageal tract, which enhances the sensitivity of the imaging system.

We also made significant progress in the automation of the SL-image acquisition process. A LabVIEW-based computer program was developed to control the optical parametric oscillator (OPO) laser (the excitation source) and the liquid crystal tunable filter (LCTF) used for wavelength selection during SL scanning. This program offers easy control of parameters for SL measurement such as start wavelength (λ_{start}), stop wavelength (λ_{stop}), step wavelength (λ_{step}) for SL scanning, and delta wavelength ($\Delta \lambda$) between the excitation and emission wavelength. Using this program, the simultaneous scanning of the OPO and the LCTF can be conveniently achieved with a click of the mouse. More importantly, the use of such a program could significantly reduce the data acquisition time down to a few seconds, which is desirable in a clinical setting.

During this reporting period, we have worked closely with our partners and co-PIs, Dr. M. Panjehpour and Dr. B.F. Overholt at the Thompson Cancer Survival Center (TCSC). Several rounds of clinical trials on human subjects using the SL imaging system have been conducted. Even with the limited number of studies from a small patient pool, we found a certain degree of differentiation between normal squamous esophageal tissue and Barrett's esophagus. We have requested a no-cost one-year extension until July 2007 in order to conduct further clinical studies using the SL-imaging system. We believe that these studies will demonstrate the great potential of SL-imaging system for the diagnosis of pre-cancer malignancies such as Barrett's esophagus and esophageal dysplasia.

ISSUES

N/A.

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PROJECT TITLE: Multifunction Prosthesis Control using Implanted Sensors

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GRANTING NIH INSTITUTE/CENTER: National Institute for Biomedical Imaging and Bioengineering (NIBIB), National Institute for Child Health and Development (NICHD)

ABSTRACT

We are developing a multi-channel/multifunction prosthetic hand/arm controller system capable of receiving and processing signals from up to sixteen Implanted MyoElectric Sensors (IMES). The appeal of implanted sensors for myoelectric control is that EMG signals can be measured at their source providing relatively cross-talk free signals that can be treated as independent control sites. Therefore the number of degrees-of-freedom that can be simultaneously controlled and coordinated in an externally-powered prosthesis will be greater than with surface EMG or mechanical control sites. From a hardware perspective we have got the system working from end-to-end. We are able to record EMG using implant electronics that are powered by an external coil and with this same coil receive the EMG signals transmitted by multiple implants. These signals are able to be decoded and displayed on a computer screen. In other work we have been exploring the issue of intra-muscular signal independence and the ability of subjects to control them.

STATUS OF RESEARCH AND PARTNERSHIP

We are developing myoelectric sensor capsules **(Fig. 1)** that can be chronically implanted into the residual muscles of an amputee's arm. By localizing the points at which myoelectric activity is detected, these points can be treated as independent control sites with minimal cross-talk. Consequently, the number of degrees-of-freedom that can be simultaneously controlled and coordinated in an externally powered prosthesis will be increased in comparison with surface EMG sites, while obviating the problems of tapping into cut motor control nerves. These sensors receive their power, digital addressing, and command signals from an external transmitter/receiver coil worn by the patient. The external coil required for the inductive link is laminated into the prosthetic socket such that this coil will encircle the implanted electrodes (**Fig. 2**). Each implanted sensor acts as an intramuscular electrode to detect the electrical activity generated as a by-product of normal muscle contraction. The implants transmit these muscle signals, or myoelectric (EMG) signals, over a shared transcutaneous magnetic link.

Each sensor's electronics and associated circuitry is to be housed in a RFB BION® hermetically sealed package provided by the Alfred Mann Foundation (AEMF)[1]. A major attraction of the BION® technology is that the hermetically sealed ceramic capsule and electrodes necessary for long-term

[1]Alfred E. Mann Foundation, Valencia, CA

survival in the body have previously been granted FDA IDE approval for use in Functional Electrical Stimulation applications. Furthermore, no wires are required to be surgically threaded down the arm. No wires are required to penetrate the skin.

Our system has achieved a number of important milestones this year: We achieved end-to-end operation of the system with implant electronics in their correct physical size for use in the AMF capsules. We have been able to place 3 implants in a magnetic coil and read EMG signals from them. Getting the system to work from end-to-end is important as it allows us to be able to query the implant electronics and get answers back concerning system state so that we can solve some remaining issues in the design. In a parallel effort working with the Alfred Mann Foundation we were able to slot dummies of our implant integrated circuit into their production line and get fully assembled and sealed capsules. We are working now to eliminate the remaining bugs on the integrated circuit before combining these two efforts to get a fully functioning implants in AMF capsules.

ISSUES

All in all our partnership has functioned very smoothly. The biggest issues we have faced has revolved around getting payments to subconctractors in a timely fashion due to the large administrative overhead associated with the University purchasing and accounting systems – this turns out to have a longer lead times then anticipated associated with it. But that said, our team although spread across multiple institutions, is small and the members communicate frequently with each other so issues like this are easily resolved.

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PROJECT TITLE: Implantable total artificial lung

PARTNERS' NAMES AND AFFILIATIONS:

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

ABSTRACT

Lung transplantation is very successful, but limited by availability of donors during the short window between listing and fatal lung failure. Extracorporeal Life Support, (ECMO)can replace lung function for weeks , But ECMO is too complex and invasive to routinely extend the window for transplantation. Another limitation to transplantation is that many potential lung donors are not accepted because borderline lung function might prove fatal in the postoperative period without mechanical support. An implantable prosthetic lung which could function for 3-6 months would solve both of these problems, just as the ventricular assist device has been applied to cardiac failure and transplantation.

In 2001 we began a ten year project to develop a clinical implantable total artificial lug (TAL).We were awarded a bioengineering research partnership (BRP) grant to design and test an implantable total artificial lung through the prototype stage. We have completed the goals for the first five years of that project . Specifically we have developed computer models of device gas exchange and hemodynamics with pulsatile flow to apply to TAL design. We have designed , fabricated , and tested devices, resulting in a prototype (the "Biolung") which meets the specifications for a functional TAL. We developed a servoregulation system to control CO2 clearance by regulating the sweep gas flow. We have routinely implanted the TAL into healthy sheep for 7-30 days, allowing us to identify the remaining problems which must be solved before clinical trials (air embolism, anticoagulation and thrombosis, ideal mode of attachment and control of blood flow, and durability in 30 day application).

With support from renewal of the BRP, we now propose to solve those problems , with the goal of proceeding to clinical trials in five years . Our team of research surgeons, bioengineers, and industry has completed the first half of this project and is ready to bring it to completion.

STATUS OF RESEARCH AND PARTNERSHIP

Computer modeling has been completed, such that new configurations and blood flow patterns can be tested on the model, revised, then applied to device design. This is an important

step, because the goal of preventing air embolism depends on the use of a new membrane which must be incorporated without compromising the specific high flow, low resistance, low impedance design which has evolved to solve the physiologic limitations of right ventricular function.

Membranes used for the development of the prototype are woven microporous hollow fibers.Density, blood distribution, and gas exchange in a defined housing allow total perfusion by the right ventricle, with or without a compliance chamber. However these fibers can leak air into the circulation if the blood perfusion pressure drops below the gas phase (atmospheric)pressure. The new fibers made by Medarray inc are solid silicone rubber without micropores.This will solve the first limitation to clinical trials.

Thrombosis in the device is controlled by continuous heparin infusion, but in clinical application our goal is no systemic anticoagulation. We are addressing this by developing plastic surfaces that elute nitric oxide in specific, continuous controlled amounts. This prevents platelet adhesion and activation, simulating the normal endothelium. Preliminary studies in rabbits and in vitro show that NO release can be controlled, platelet adhesion and thrombosis prevented, without compromising gas exchange.

Our studies have focused on PA to LA connection, with TAL perfusion by the right ventricle. This attachment mode will be used for patients with severe pulmonary hypertension, but has the disadvantage of possible systemic thromboembolism. Other groups have shown that PA-PAconnection would be limited by human PA anatomy, in addition to adding to the pulmonary vascular rersistance.RA to PA attachment requires a pump, which we wish to avoid. We have conducted pilot trials of a cavo-right atrial attachment mode which would allow complete gas exchange support and avoid systemic embolism without a pump. We plan full evaluation of this technique in the next year.

We have a self imposed goal of routinely successful 14-30 day implantation in sheep before progressing to clinical trials as a bridge to lung transplant. We can meet that goal now with systemic anticoagulation and suction on the gas phase to avoid air embolism. Following the research steps above, we expect to reach clinical trials within five years.

The partnership has been very successful. The next steps will use the models and methods from the bioengineering group to fabricate the definitive device for the remaining studies.

ISSUES

As we proceed to the final animal studies, the models from the bioengineering group will be essential for modifications of the design. However the majority of the studies are animal testing of the physiology and thrombogenicity. The budget limitations specified for the renewal application put the emphasis on bringing the final device to clinical application.

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

PARTNERS' NAMES AND AFFILIATIONS:

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Kai Thomenius, Ph.D., Andrea Schmitz, MS, Jeff Eberhard, Ph.D., GE Global Research, Niskayuna, NY

GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI)

ABSTRACT

The U of M and GE partnership plans to develop, test, optimize, validate, and translate toward the clinic a hybrid breast imaging system for diagnosis and eventual screening. Aim 1 will combine 3D ultrasound (US) and digital mammography (DX) units on a shared breast compression unit. Each compression will yield a physically and temporally registered image set. 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 a high speed, low vibration, wide angle, high sensitivity digital x-ray tomosynthesis mammography (DTM), as well as US (ultrasound) color flow and elasticity imaging.

STATUS OF RESEARCH AND PARTNERSHIP

Digital X-ray/Ultrasound Breast Imaging System: The digital tomosynthesis mammography/ultrasound (DTM/US) system from GE Global Research was installed in late fall, replacing the combined full field digital mammography and ultrasound system. The system has generally worked well, giving outstanding, well-registered, multimode images as expected from the design.

Advanced Imaging Modes: Major efforts have continued in refinement of advanced ultrasound and x-ray imaging modes which are providing evidence of substantially different information from that of the DX and US images of the core (first generation) combined system. Nonlinear Elasticity Imaging: Using the combined 3D US/Digital X-ray system or a similar stand alone US system, 2D, 3D, and crossbeam elasticity images were obtained in a breast mimicking phantom and *in vivo*. Images were correlated using 2D or 3D speckle tracking algorithms to yield axial displacements and strain images. *In vivo,* elasticity imaging was accomplished in 26 human subjects. Strain images accumulated at up to 10% mean strain clearly characterized lesions as cysts, fibroadenomas, or cancers in 11 out of 16 cases.

Vascularity Analysis: Results to date suggest that blood flow/pressure characteristics exhibit local detectable changes under varying compression conditions, possibly leading to discrimination of pathology.
Compound/Multiview Imaging: Compound B-mode images have higher SNR than conventional single view B-mode images and give better display of structures, and better image volume-based registration. Current limitations make this impractical on current trials, so speckle-reduction algorithms are employed.
Digital tomosynthesis mammography: This research prototype allows for more images and larger angular range than systems being developed for initial commercial introduction. U of M and GE are studying reconstruction algorithms and effects of image acquisition, finding that DTM image sharpness and contrast decrease with decreasing numbers of projection views (PVs) used in the reconstruction. The interplane artifacts increased with decreasing angular range of the PVs.

Advanced Image Processing

Image-based registration: Registration of partially overlapping US sweeps was tested for splicing the sweeps to form the complete 3-D volume resulted in a negligible translation error of ~0.6 mm. A study for another project was conducted on human fingers with the BRP stand-alone system for identification of the individual. US images (grayscale, compound and Doppler modes) were acquired on the same individual at different time points and stress levels. In 15 pairs of cases, all individuals were matched successfully to themselves using both image based registration and expert readers.
Multimodality CAD: Our new approach to mass detection combines 3D information from reconstructed DTM slices and from the projection views (PVs). In a study of 26 DTM cases acquired with a GE DTM prototype system at the MGH, the average FP rates were reduced by 1/2 when DTM classification was combined with information from the individual projections.

Clinical Studies: 86 women have been studied to date with a combined system, 46 in the past year. Of these, 32 were with both DTM and US on the new combined system. Reader Studies are in progress with 4 expert radiologists independently evaluating cases with US and DTM. Contrary to preliminary results with the combined US/FFDM system, in the majority of the first 10 cases the readers are, on average, ranking the visibility of masses on the clinically acquired freehand US images as better than the automated US. The question about the utility of multiplanar imaging so far has wide inter-reader variability, with DTM usually being preferred over clinical mammography.

ISSUES

The DTM system from GE Global Research is the first of it's kind with: a rigid column and base to reduce vibration artifacts; special x-ray tube, mechanical design and detector electronics to enable faster acquisition with almost twice as many view angles as the first generation DTM. Even so, with a new, more sensitive detector, the dose is the same as the first generation system at MGH -- one DTM volume = ¾ the dose of a two-view mammogram set. The DTM images appear outstanding, with masses standing out very clearly compared with their appearances in many of the mammograms. The US image quality is quite acceptable when the acoustic coupling is performed successfully, but conduct of the ultrasound is more difficult with the DTM unit, which currently has a bulky patient shield for protection from radiation and the rapidly moving tube. This type of tomosynthesis mammography should improve diagnostic accuracy, particularly in the dense breast. US should still have some role in lesion characterization. The combined system or a standalone US system in similar geometry might well be useful in diagnostic patient flow to help assure identification of the same lesions in x-ray and US.

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PROJECT TITLE: Engineering Aspects of Liver Support Systems

PARTNERS' NAMES AND AFFILIATIONS:

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GRANTING NIH INSTITUTE/CENTER: NIDDK

ABSTRACT

In spite of many advances in liver transplant surgery, an increasing number of patients with terminal liver disease are dying while awaiting a transplant. Consequently, further advances in the storage of donor livers, as well as alternative replacement options are needed. A very promising area of research and development is in the development of engineered solutions to the problems of liver support. However, efforts undertaken within a single discipline are hampered by the complexity of both the engineering and biological aspects of such projects. This proposal constitutes a partnership between bioengineers and biologists with the goal of combining their expertise to devise improved methods of liver support via bioartificial livers and improved preservation of donor livers via machine perfusion preservation (MPP). The partnership encompasses three inter-related projects. The first project focuses on deliver of oxygen and other nutrients to the cell in in vitro systems such as the bioartificial liver. The approach involves the modification of the support matrix to facilitate enhanced mass transport. The second project addresses the hypothesis that improved bioartificial liver function can be attained by providing a more physiological combination of cell types in the support device. Specifically, we will investigate the relationship between Kupffer cells and hepatocytes in maintaining prolonged hepatic-specific function in culture. The final project focuses on development of methods for optimization of microvascular perfusion and oxygen delivery in pump perfused livers. This project uses a combination of intravital microscopy and mathematical modeling. In all of the projects, engineering and biological approaches are combined to address focused, clinically relevant problems. Moreover, the unique environment that supports the partnership will maximize the potential for success in this interdisciplinary approach.

STATUS OF RESEARCH AND PARTNERSHIP

Our BRP partnership is nearing the end of its fifth year of funding. Although our funding Institute, NIDDK is no longer participating in the BRP program thus precluding submission of a competing renewal, we consider our partnership to be a great success. In addition to providing

data for approximately 15 publications, the partnership has resulted in additional NIH funding, provided a springboard for the establishment of a Center for Biomedical Engineering Systems at the University of North Carolina at Charlotte and provided the intellectual property that formed the basis for the incorporation of a company, HepatoSys Inc cofounded by the PI and one of the partners (C. Lee). HepatoSys has now submitted on STTR and one SBIR proposal to the NIH, both of which are scheduled for funding.

In subproject 1 we sought to determine whether nutrient delivery in a model bioartificial liver device could be enhanced by modification of the support matrix for the hepatocytes. Our results have shown that the effective diffusion distance for oxygen (but not glucose) is highly significantly increased with enhanced matrix (ECM) without or in the presence of respiring hepatocytes. This result raised the question of whether the enhanced oxygen delivery might result in increased oxidative stress. To the contrary, matrix with a high concentration of enhancement beads showed a decreased level of reactive oxygen production, a more normal NAD/NADH ratio and improved cell viability in short term studies. Long term culture studies showed that cells cultured in a sandwich culture with enhanced ECM showed better preserved cellular antioxidant systems as indicated by increased levels of catalase and glutathione reductase. Thus enhanced oxygen delivery through enhanced transport improved hepatocyte metabolic status as well as antioxidant systems. These findings are now being extended by testing the effect of enhanced oxygen carrying capacity of the medium using perfluorocarbon. These studies are funded by an additional NIH grant to Dr. Coger.

Subproject 2 investigated the effect of micropatterned coculture on maintenance of hepatocyte function in culture. We developed a method for manufacturing a micropatterned surface for coculture of hepatocytes and Kupffer cells. We found that the micropatterned culture was superior to random coculture and that a 10:1 hepatocyte to Kupffer cell ratio produced an optimal effect on hepatocyte function and preserved Kupffer cell ability to produce IL-6 in response to an endotoxin challenge.

Subproject 3 investigated the use of hypothermic machine perfusion in preservation of livers for transplantation. Our results have shown that damage to endothelial cells is responsible for failure of livers following long-term hypothermic perfusion. This damage can be ameliorated with the use of oncotic support. On the other hand, we found that short-term (5 hour) hypothermic perfusion substantially restored energy stores and overall function in livers taken from a rat model of nonheart-beating donors (30 minutes warm ischemia prior to preservation). In a nonheart-beating model that resulted in 100% mortality in rats transplanted with livers preserved by simple cold storage for five hours, hypothermic perfusion for five hours prior to transplant yielded 84% survival. The PI and Dr. Lee have now founded a company (HepatoSys Inc) with the goal of further developing this technology to bring it to clinical trials. HepatoSys has one STTR being processed for funding which will scale up the system to evaluation of the process in isolated perfused pig livers and an SBIR also being processed for funding which focuses on optimization of the perfusion solution for hypothermic perfusion.

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PROJECT TITLE: Bioengineering Design of Artificial Blood

PARTNERS' NAMES AND AFFILIATIONS:

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

ABSTRACT

Our objective is the design and development of artificial oxygen carrying plasma expanders (OCPEs) based on the modification of the hemoglobin molecule aimed at formulating an oxygen carrying fluid that has comparatively high viscosity, high affinity for oxygen, high oncotic pressure and that is economic in the use of hemoglobin, i.e., is effective with a minimal concentration of hemoglobin. These goals are being achieved via surface attachment polyethylene glycol (PEG) to the hemoglobin (Hb) molecule. Variables in PEG attachment include length and number of PEGs, bifurcations and bending moments. On biophysical considerations each variant has different solution properties, that may affect oxygen binding. A PEG formulation has been optimized in terms of cost, biological efficacy, COP, vasoinactivity, vascular retention and viscosity. Physiological research in the microcirculation was performed for further understanding the foundation of tissue oxygenation and is used to explore how alterations of blood physical properties affect tissue oxygenation and tissue survival in extreme hemodilution and shock. This program emphasizes the comprehension of the mechanism necessary for a stable balance between NO scavenging by molecular Hb in solution and the production of EDRF by shears stress

dependant mechanisms

STATUS OF RESEARCH AND PARTNERSHIP

The initial activity was implementation of a research and development plan leading to the design of a product that can be manufactured and delivered at a cost competitive with blood. The problem of effectiveness was addressed by establishing a control baseline relative to existing products. Microvascular tests were made at UCSD to determine the transport properties of an oxygen carrying bovine molecular hemoglobin solution manufactured by Biopure Inc. marketed for veterinary applications. Analysis of the effectiveness of this product was made by determining functional properties of the microcirculation during extreme hemodilution and shock and comparing this with similar procedures carried out with conventional carrying plasma expanders. We found that this molecular hemoglobin based product provides no functional improvement over that attainable with conventional colloidal plasma expanders, supporting the need for a radically new approach which was attained with MaleamidePEG-Hb. The efficacy of this hemoglobin modification was tested in the microcirculation of the hamster window model, in hemorrhagic shock experiments in a rat model, and in a swine hemorrhage protocol carried out in collaboration with the Swedish Defense Establishment (FOI) in Stockholm, Sweden. Experimental studies show that Mal-PEG-Hb is superior to conventional plasma expanders and blood in maintaining functional capillary density, acid base balance, perfusion and survival. This product has been submitted for evaluation in terms of toxicity, biodistribution, intravascular retention time and systemic cardiovascular effects by Sangart Inc., of San Diego, Inc., who developed production facilities and organized Phase 1 and Phase 2 clinical trials that were successful. The material is now in Phase 3 clinical trials in Europe, and Phase 2 clinical trials in the US.

ISSUES

No issues.

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PROJECT TITLE: Ophthalmic Imaging Using Adaptive Optics and OCT

PARTNERS' NAMES AND AFFILIATIONS:
Scot Olivier (Lawrence Livermore National Laboratory)
Donald T. Miller (Indiana University)

GRANTING NIH INSTITUTE/CENTER: National Eye Institute (NEI)

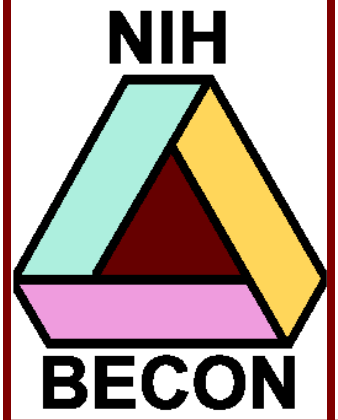
ABSTRACT

The purpose of this BRP is to develop and evaluate new optical instrumentation that will permit unprecedented three-dimensional, *in vivo,* imaging of the human retina at the cellular scale. 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 third year of funding. In previous years we used AO correction of ocular aberrations in our OCT cameras. While AO correction has successfully reduced the degrading impact of ocular aberrations, no retinal imaging cameras have provided sufficient correction to yield diffraction-limited imaging (Strehl > 0.8) across large pupils (≥ 6 mm). The correctors available still do not provide high dynamic range and spatial fidelity to fully compensate both low- and high-order aberrations of the eye. To overcome this barrier, the BRP developed a novel AO system that cascades two deformable mirrors.

The AO-OCT system was tested with a superluminescent diode light source enabling axial resolution of 6.5 μm in retinal tissue. The optical design used a cascade of afocal telescopes (created by pairs of spherical mirrors) to conjugate the eye's pupil plane with the X and Y scanning mirrors, the two wavefront (WF) correctors (deformable mirrors) and a Hartmann-Shack WF sensor (which uses a fraction of the OCT imaging light for WF reconstruction). The deformable mirrors (DM) included: AOptix Technologies, Inc 35-element bimorph and Boston Micromachines Corporation 144-element micro-deformable mirror. Due to its maximum stroke of ±32 μm and small number of actuators, the bimorph mirror (DM1), provides better performance for correction of relatively large, low-order aberrations. On the other hand, the Boston Micromachines DM (DM2) offers superior performance for higher-order aberrations (however, with limited dynamic range). The operation of the AO control system, consisted of



Project Summaries Coor

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PROJECT TITLE: Cold Neutrons for Biology and Technology

PARTNERS' NAMES AND AFFILIATIONS:

GRANTING NIH INSTITUTE/CENTER: National Institute for Research Resources (NCRR)

ABSTRACT

(From Grant) The Cold Neurons for Biology and Technology (CNBT) partnership consists of investigators from six universities, the National Institute of Standards and Technology (NIST), Los Alamos National Laboratory (LANL), and the NIH committed to the development of advanced neutron scattering instruments for studies of membrane systems at the NIST Center for Neutron Research (NCNR). Specifically, these instruments will be devoted to basic and applied studies of membranes and macromolecules in membranes, and to membrane-based technologies that include studies of protein complexes with relevance to bioengineering. The instruments, consisting of a fully dedicated biological advanced neutron diffractometer/reflectometer (AND/R) and a 30-meter small-angle neutron spectrometer (SANS) dedicated 10 percent to biology, will provide combined advantages and capabilities not currently available in the United States. During the first two years of the project, the AND/R, which has already been designed with the aid of a planning grant from the NSF, will be constructed and commissioned and an existing world-class SANS instrument will be optimized for membrane research. At the same time, a high-performance computer system will be put in place to support the concerted use of neutron diffraction and molecular dynamics methods in order to deduce 3-D structural information from 1- or 2-D diffraction data. Finally, new laboratory space adjacent to the neutron instrument hall will be renovated and equipped to serve the special needs of the partnership and other biological users. Concomitantly, research and technical staff will be recruited. Some early progress on the tasks of the partnership will be achieved using the existing non-optimized SANS and the existing reflecting/diffraction instruments at the NCNR during these two years. The development of the new membrane-optimized instruments will be driven by distinct experiments inspired by the research programs of the CNBT team. The expertise of the team members, drawn from departments of chemistry, physiology, cell biology, and physics, includes membrane diffraction, small angle neutron scattering, membrane molecular dynamics (MD), biosensors, and biomaterials. Linking neutron diffraction measurements to MD simulations of biomolecular structure is an important objective of the team. We foresee a future when computer simulations will allow three-dimensional detail to be inferred routinely from 1- and 2-dimensional neutron and X-ray data.

STATUS OF RESEARCH AND PARTNERSHIP

ISSUES

None

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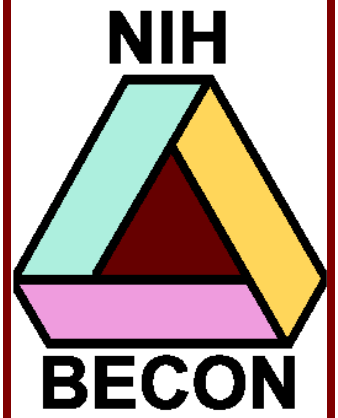

Project Summaries

Continued

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PROJECT TITLE: Breast CT Scanner for Earlier Cancer Detection

PARTNER 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)
UC San Francisco (San Francisco, CA)
Stanford University (Palo Alto, CA)
Hahnemann University (Philadelphia, PA)
University of Arizona (Tucson, AZ)

GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB), previously National Cancer Institute (NCI)

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 optimally image the breast in conventional CT scanners without significant chest artifact and reduced resolution. Therefore, in this Bioengineering Research Partnership project, we have teamed with scientists from around the country to design, build, and test a CT scanner designed to image the breast. The team comprised of medical physicists, physicians, mechanical and electrical engineers, and breast cancer advocates has designed and constructed a scanner capable of scanning the breast in less than 15 seconds. The scanner includes a breast immobilization system (acrylic cylinders), a breast CT table, fast reconstruction algorithms and computers, and a computer workstation customized for efficient viewing breast CT images. The scanner has been built and tested at UC Davis over the past 3 years (9 specific aims). Ongoing optimization continues as part of refining acquisition and reconstruction algorithms. Evaluation of phase I clinical trial results (2 specific aims) has provided valuable data that is used for scanner refinement and optimization of clinical image evaluation. Subsequently, the scanner has been 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 metastases 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 BRP partnership is proceeding with what we view as excellent progress. An early prototype breast CT scanner in the PI's laboratory has facilitated investigation of a significant number of design and scientific issues associated with the breast CT project. These investigations have provided invaluable information that has been crucial in the design of the breast CT scanner that has been constructed under the auspices of this BRP funding. This scanner provides a broad evaluation platform for more advanced acquisition and analysis of breast CT data. In addition the second scanner also has the capacity to accommodate multi-modality breast imaging technology (dual energy, PET, Ultrasound funded by complementary awards). The PI has an active on-going dialog with the co-PIs at partner institutions (particularly those in California) with consultative visits on numerous occasions. There was a meeting of all the collaborators and consultants in February 2005 to review the project progress and provide critical input to the design and testing of the scanner. The consultants comprise, what is in effect, an external advisory board with group meetings functioning much like a site visit.

ISSUES

Thus far we are well along the path of accomplishing the specific aims of the BRP. Thus, we have no major issues or problems to report. While we have maintained strong collaborative relationship with the partners within the same time zone (all in California), our East Coast partner (at Duke University) is not in the loop as much as desirable. We are striving to improve this situation.

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PROJECT TITLE: Micromechanical Device for Intracochlear Drug Delivery

PARTNERS' NAMES AND AFFILIATIONS:

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Massachusetts Eye and Ear Infirmary, Boston MA: Sharon Kujawa, Michael McKenna, William Sewell, Zhiqiang Chen

GRANTING NIH INSTITUTE/CENTER: National Institute on Deafness and Other Communication Disorders (NIDCD)

ABSTRACT

Our goal is to develop an implantable long-term drug delivery system for treatment of inner ear disorders and prevention of sensorineural hearing loss. We envision a versatile device capable of delivering multiple simple and complex molecules over long periods of time, with capability to control sequence and rate of delivery. Such a device could have immediate application for treatment of idiopathic, ototoxic and inflammatory conditions affecting the inner ear, including autoimmune inner ear disease, cisplatinum-induced ototoxicity, and possibly Meniere's disease. In addition, a wide spectrum of other degenerative inner ear disorders may soon be amenable to treatment with such a device, including idiopathic, genetically-based, and age-related progressive sensorineural hearing losses. Combined, these disorders affect millions of individuals in the United States. Over the last three years, we have developed a working collaboration between a group of micromechanical engineers from Draper Laboratory and a group of auditory scientists and otologists at the Massachusetts Eye and Ear Infirmary. The Draper group has established success in development of implantable drug delivery microsystems. The MEEI group has extensive experience in the physiology and pharmacology of the inner ear and in otologic surgery. Together we have worked to develop design concepts and have produced a perfusion system for testing and modeling such a device in animals. Our next steps are to refine our working prototype and to test critical design parameters in chronic studies in animal models. The design concept includes a device which fits within the mastoid cavity of humans, a high efficiency electronically controlled pump used to recirculate perilymph, a catheter inserted into scala tympani through a cochleostomy adjacent to the round window, a valved drug reservoir, externally programmable controls for delivery of concentrated bioactive compounds, and sensors for detecting and transmitting flow information. Towards these goals, our specific aims are: (1) Develop biocompatible microfluidic interface to the inner ear suitable for chronic, continuous recirculation of and perfusion of compounds into perilymph, (2) Develop low-power micropump and integrated micromachined fluid control system, (3) Establish surgical procedure and determine device form factor constraints for implantation in the human mastoid cavity**,** (4) Implement implantable drug storage reservoir, filling, and release mechanism appropriate for a range of candidate therapies, (5) Integrate low power control electronics and telemetry design, and specify power source, (6) Evaluate safety and efficacy in preliminary animal experiments, using prototype devices to deliver bioactive compounds to the inner ear.

STATUS OF RESEARCH AND PARTNERSHIP

The BRP project began in March of 2006 and we are focusing on three areas during this first phase of the program, including establishment of a safe and robust surgical procedure, development of the hardware for the successive generations of animal studies, and the identification and pursuit of the highest priority clinical applications. Chronic and acute experiments in which test compounds are delivered to the cochlea of the guinea pig are now showing that hearing is preserved across the procedure, and that the surgical procedure is safe, reproducible and robust. A reciprocating pump system is being used to perfuse drugs into the cochlear perilymph through a single surgical hole in the scala tympani. Studies with artificial perilymph perfusion demonstrate little change in hearing, notwithstanding transient fluid effects in the middle ear, and therefore the surgical procedure is a robust means to monitor the pharmacokinetics of compounds using the Place Principle. Glutamatergic antagonists that act only on the inner hair cells have been perfused using this procedure, and the influence of these compounds on Compound Action Potentials (CAPs), which are controlled only by the inner hair cells, can be monitored as a function of frequency to track the advance of the test compound through the cochlea. Diffusion models based on the pioneering work of Salt et al (Washington Univ.) have been adapted to our drug delivery system and are now being used to establish the delivery parameters for long-term perfusion to the cochlea.

The preliminary prototype drug delivery device utilizes commercial components housed in a head-mounted pod for acute and chronic guinea pig studies. We have completed the design of a compact wireless control system for the next generation device, which is powered by a set of batteries to enable operation over periods of several weeks. The programmable controller enables the delivery protocol to be monitored and modified remotely, so that longer-term drug perfusion studies can be conducted across a wide range of experimental conditions. Combined with the cochleostomy procedure that has now been established, these studies will be used to support investigations of the efficacy and safety of compounds as well as for more fundamental studies of the molecular mechanisms of inner ear disease. In parallel with the construction and testing of the head-mounted prototype, development of the fully implantable device using a micropump and flow controller is continuing, with the goal of completing the development of this system by the third year of the program.

The principal clinical application of this program is the treatment of sensorineural hearing loss, which affects 27 million Americans and represents the leading birth defect in the United States. The availability of this device will provide an avenue for long-term therapy for this devastating condition but will also enable discovery of new compounds for the treatment of this and other disorders. In addition to the sensorineural hearing loss application, we have identified numerous other clinical targets for this drug delivery device, and are currently establishing interactions with research teams addressing vestibular disorders such as Menière's disease, tinnitus, and protection from ototoxicity for patients receiving cisplatin-based chemotherapy. New and exciting applications are emerging from our interactions with these groups, and our goal is to address the spectrum of opportunities for drug delivery to the inner ear using this new platform.

ISSUES

The project is off to an excellent start, with no issues encountered to date. The Draper Laboratory and Massachusetts Eye and Ear technical teams have been working together for several years prior to the start of the BRP, and are seamlessly integrated towards the achievement of the project goals.

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PROJECT TITLE: Dynamic Signal Processing Analyses of Neural Plasticity

PARTNERS' NAMES AND AFFILICATIONS:

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

GRANTING NIH INSTITUTE/CENTER: National Institute of 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 Whales) 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 recorded 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 through 4 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 (Matthew A. Wilson, MIT). We have completed 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 that have been contrasted with simultaneously measured changes in CA1 (Specific Aim A-B). We have applied analytical techniques that have been developed in the Brown laboratory for

characterizing the dynamics of these changes. We are now collecting additional data and anticipate final completion of the work this year. Behavioral studies and behavioral hippocampal recordings have been completed on a new line of DG-specific NMDAR-KO animals (Specific Aim C) and this work is being prepared for publication. We successfully identified changes in hippocampal receptive field properties that related to specific learning and memory behavioral deficits involving performance in novel environments or following novel cue manipulations.

Specific Aim 2: Dynamic Analysis of Information Encoding Within the Hippocampus and Adjacent Regions of the Medial Temporal Lobe (Wendy A. Suzuki, NYU). This past year our progress has centered on two major projects. First, we have continued our detailed comparisons between the dynamic learning-related activity in the perirhinal cortex and hippocampus. Developments during the past year include a more detailed analysis of changes in selectivity with learning as well as a detailed characterization of the patterns of response profiles of the learning-related cells. These analyses revealed that while the learning-related changing cells in the hippocampus can either increased or decrease their selectivity with learning, perirhinal changing cells only increased their selectivity with learning suggesting a difference between these areas. In contrast the response profiles of both these areas within the trial were very similar, showing changes either in the scene, delay or response period of the task. A second area of progress has been in increasing our population of learning-characterized cells in the entorhinal cortex. We now have over 80 well isolated cells characterized that have been recorded from the entorhinal cortex using either single electrodes or tetrodes. We have completed a first pass analysis which reveals both selectively responding cells in this area as well as changing cells. We are in the process of analyzing the data in more detail and comparing it with the data from both the hippocampus and the perirhinal cortex.

Specific Aim 3: Dynamic Signal Processing Methods for the Analysis of Neural Plasticity (Emery N. Brown, MGH/HMS): We have successfully developed a maximum likelihood algorithm to jointly estimate the relationship between spiking activity and behavior based on a joint model of the two. We have successfully used the algorithm to analyze simultaneously recorded binary responses and neural activity collected on the location-scene association protocol (Okatan et al., 2006).

Significance

Specific Aim 1 We have demonstrated the ability to identify novel receptive field dynamics and characteristics that can be related to behavioral performance through the application of genetic manipulations. We have also demonstrated the ability to successfully monitor the simultaneous dynamics of receptive field characteristics in 3 hippocampal subregions (DG, CA3, CA1) and identify novel receptive field relationships between them as well as their dynamics during training.

Specific Aim 2 Our goals for the next funding period include expanding the tetrode recording to both multiple locations within the entorhinal cortex as well as paired recordings across different MTL regions. We will also continue or analyses focusing on characterizing the similarities and differences in learning-related signals across these medial temporal lobe areas.

Specific Aim 3 This finding is important because the relationship between behavior and neural activity is assessed based on a joint model rather than estimating a model for each process and then comparing them through a correlation analysis.

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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Department of Physical Therapy: Stuart Binder-Macleod, Darcy Reisman, Katherine S. Rudolph, John P. Scholz

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

ABSTRACT

Stroke is one of the leading causes of functional disability among American adults. The effects of post-stroke hemiparesis include reduced muscular strength and endurance as well as diminished mechanical work output and altered muscular activation patterns during gait. These combined effects lead not only to reduced mobility, but may also contribute to increased injuries from falls in this population (Nyberg et al 1995).

The overall 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.

This project combines the resources of the mechanical engineering and physical therapy departments at the University of Delaware to develop and integrate a robotic gait rehabilitation device with an intervention strategy and functional electrical stimulation (FES) to assist hemiparetic patients during gait. This will be combined with minimally invasive intervention strategy based on musculoskeletal modeling and gait simulation. The five-year goal of this project is to assist patients with CNS dysfunction to produce improved or optimized movement patterns that will maximize postural stability, minimize musculoskeletal injury (e.g., arthritis) during movement, develop a more natural looking gait, and minimize metabolic energy consumption during movement. This four-phase optimization protocol will be realized through an integration of musculoskeletal modeling, robotic assistance, functional electrical stimulation, and neuromuscular training. The specific task we will study will be 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. This is being accomplished through biomechanical modeling using gait analysis and electromyographic data. Aim 2: Develop the methods and equipment necessary to implements the optimization of locomotion in patients with stroke. We are achieving this through the use of a robotic device and an electrical stimulation system. Aim 3: Test the feasibility of the use of the 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. In the first phase of this project the focus has been on individuals with stroke exhibiting hemiparetic leg impairment however the technique used are generalizable to a variety of neurological impairments.

STATUS OF RESEARCH AND PARTNERSHIP

The Partnership is doing very well with all teams currently working closely to implement the intervention strategy. Patients are currently undergoing training sessions with the combined biomechanical modeling, FES, and robotics tools while the intervention strategy is also being optimized for motor learning with visual feedback.

Biomechanical modeling: We have developed a biomechanical model of the ankle and knee to use for this project. This model characterizes the morphology and force generating capacity of the musculature spanning the ankle. The model allows us to estimate muscle forces from EMG signals during dynamic tasks and we use the 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. This is then input to an FES controller and applied to the patient during gait.

FES: The major activities for the past two years have continued the development of the hardware and software interfaces we now use to integrate the feedback from the robot to produce real time desired muscle force outputs from the stimulation profiles provided by the neuromusculoskeletal model. The stimulation provided by the FES group augments the voluntary activation of the patient. The amount of stimulation delivered to the patient is estimated by a force and motion model which we have developed over the past year. We use a controller to modulate the pulses based on the information provided by the robot and predicted from the neuromusculoskeletal model and we implement our FES stimulation protocols including feedback on National Instrument's CompactRIO system.

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 assist 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 which help guide and describe a desired gait pattern to the user. This device is being developed with 'virtual walls' which can be adjusted to help guide the patient towards an optimal gait pattern using a progressive learning strategy.

ISSUES

There are no administrative issues that have arisen in regards to our partnership. Over the past year the integration of all research groups involved in the project has allowed us to implement our intervention and teaching strategy. As such we have now launched a gait training study with stroke patients.

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PROJECT TITLE: Optical Biopsy Using MEMS Technology

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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI)

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 PARTNERTSHIP

In the fourth year of this project, we continue to make progress on the functional OCT (F-OCT) technology and endoscopic 3-D MEMS endoscopic OCT probes.

We have developed a Fourier domain F-OCT (FD-F-OCT) system using a sweeping light source. FD-OCT significantly increases the imaging speed and sensitivity over the conventional time-domain system, which makes it possible to obtain *in vivo* 3-D imaging. One of the limitations of FD-OCT is the mirror imaging ambiguity. An innovative system design and imaging processing algorithm were developed that removed the mirror image in Fourier domain OCT. The sweeping source based FD-OCT has the advantage of simple design, low cost, and compact.

Following the successful demonstration of high resolution second harmonic OCT, we have developed a Fourier domain second harmonic optical coherence tomography (FD-SH-OCT) system that significantly reduces the imaging time. SH-OCT combines the molecular sensitivity of second harmonic generation with sectioning capability of OCT to obtain *in vivo* images with molecular contrast. Because SH-OCT uses 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 has been applied to image biological tissues of the rat-tail tendon. Highly organized collagen fibrils in the rat-tail tendon could be visualized in recorded images.

We have developed several endoscopic probes that can take advantages of high speed offered by FD-OCT for 3-D imaging. A fiber-optic bundle based OCT probe is developed. This novel OCT imaging approach eliminates any moving parts in the probe and has a primary advantage for use in extremely compact and safe OCT endoscopes to image internal organs and great potential to be combined with confocal endoscopic microscopy. In addition, 3-D endoscopic OCT system based on a dual axis MEMS mirror was demonstrated. The diameter of the MEMS mirror was 1.2 mm and both axes were capable of scanning up to 20° (optical) with excellent linearity. The dual axis scanning 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 FD-OCT system. The MEMS mirror provides high-speed, high resolution 2-axis scanning while occupying a very small volume with extremely low power consumption.

Finally, we have finished our testing of the probe. Clinical trials on imaging and evaluating cancers in the GI tract are planed in Fall 2006.

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

ISSUES

One of the Co-PI, Dr. Norman Tien, moved from UC Davis to Case Western University. This move delayed the project on the 3-D MEMS probe assembly. However, this problem has been resolved. The second 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. Although the original focus of the partnership is the development of endoscopic OCT using MEMS based probes for cancer diagnosis in the GI tract, currently, we have one system set up in the UCI Medical Center that has been used for imaging cancers in larynx and upper airway, etc. However, it is difficult to accommodate most of these requests with only one system developed from this grant.

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PROJECT TITLE: Prevention of Hemodialysis Vascular Access Stenosis

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Dr. Michael Kirby (Scientific Computing & Imaging Institute, University of Utah)
Dr. Donald Blumenthal (Dept. of Pharmacology & Toxicology, University of Utah)
Dr. Alfred K. Cheung (Dept. of Medicine, University of Utah)
Dr. Dennis Parker (Dept. of Radiology, University of Utah)

GRANTING NIH INSTITUTE: National Heart, Lung and Blood Institute (NHLBI)

ABSTRACT (From grant renewal application in 2005)

Stenosis caused by neointimal hyperplasia (NH) often occurs focally at the anastomoses of arteriovenous (AV) grafts used for hemodialysis, leading to thrombosis and occlusion. This is a competitive renewal re-submission for a project to develop novel sustained drug delivery systems that could be injected percutaneously and allow local delivery of anti-proliferative drugs to prevent graft stenosis. The Bioengineering area is “Clinical Medicine, Therapeutics and Drug Delivery”. Using a unique polymeric drug depot (ReGel) to deliver paclitaxel perivascularly, we have shown that this approach is feasible and effective in a canine model. We have now perfected a porcine model and demonstrated the sustained, quantifiable delivery of dipyridamole from the perivascular depot into the vessel walls over weeks.

The 4 specific aims in this renewal are to: (1) optimize sustained-release polymer gel systems for local delivery of specific anti-proliferative drugs based on each drug's physicochemical, pharmacokinetic and pharmacodynamic properties; (2) develop and validate finite element models to predict the long-term pharmacokinetics of drugs administered perivascularly using sustained-delivery systems at the anastomoses of AV grafts; (3) identify drugs that are safe and efficacious in preventing stenosis at the graft anastomoses when administered using sustained-delivery systems; (4) adapt and refine 3D imaging modalities, including magnetic resonance angiography (MRA), for more accurate quantification of stenosis progression and drug efficacy at the graft anastomoses in the porcine model.

There are 6 Leading Investigators in 5 departments: (1) R. Rathi, MacroMed, Inc. (development of polymers for drug delivery); (2) S. Kern, Depts. of Pharmaceutics and Bioengineering and M. Kirby, Scientific Computing & Imaging Institute (pharmacokinetic modeling and validation in tissues); (3) D. Blumenthal, Dept. of Pharmacology & Toxicology (characterization of in vitro drug efficacy and mechanism of action); (4) A. Cheung, Dept. of Medicine, U. of Utah (animal experiments and clinical correlation) who also serves as PI and Project Manager; (5) D. Parker,

Dept. of Radiology (MRA imaging development and 3D reconstruction of NH morphology). This multidisciplinary team, using an integrative systems approach, is essential for the development of innovative methods to solve an important clinical problem. It will also offer excellent opportunities for trainees of various disciplines to interact with each other in a collaborative manner. It is highly likely that, within this 5-year proposal, the results of this project can be applied to pilot clinical studies.

STATUS OF RESEARCH AND PARTNERSHIP

Specific Aim 1: The in vitro release kinetics of paclitaxel, dipyridamole or rapamycin from ReGel have been extensively characterized. Because of the relative hydrophilicity of dipyridamole, encapsulation of the drug into polymeric microspheres prior to mixing with ReGel was necessary in order to provide sustained release of the drug. In contrast, mixing of rapamycin, which is more hydrophobic, with ReGel alone was sufficient to provide sustained release of the drug for at least 7 weeks. Dr. Rathi at MacroMed and Dr. Blumenthal continue to be the respective leaders of the Pharmaceutics and Pharmacology partnerships responsible for this specific aim. In the past year, Dr. Fowers at MacroMed has also become more involved in developing methods of optimizing the release profile of various drugs from polymers.

Specific Aim 2: We have added two partners, Dr. Kirby (Scientific Computing & Imaging Institute) and Dr. Y. Shiu (Dept. of Bioengineering) to the Bioengineering partnership of this project, with Dr. Kirby as the co-leader with Dr. Kern. With the collaboration with these partners, we have initiated the development of high-order finite-element simulations of drug release from ReGel and its subsequent diffusion through tissue. These initial models will be adapted for each delivery system and refined and validated using data collected from in vitro and in vivo experiments. These validated models will then be used to guide the development of drug delivery systems.

Specific Aim 3: There is no change in the Animal/Clinical partnership that deals with this specific aim, with Dr. Cheung being the leader. In the last year, we found that the perivascular delivery of dipyridamole using polymeric microspheres and ReGel was ineffective in preventing myointimal hyperplasia in the porcine carotid-jugular graft model. The perivascular delivery of rapamycin using ReGel is currently being examined in the same animal model.

Specific Aim 4: Dr. Parker continues to be the leader of the Imaging partnership. Dr. T. Tasdizen in the Scientific Computing & Imaging Institute has joined our project in the last year, with the primary responsibility of performing segmentation of the magnetic resonance images. We have performed MRI on pigs with implanted grafts using a Siemen's 3T scanner and human carotid coils. In preliminary studies, we were able to successfully correlate the cross-sectional images from MRI with the histological cross-sections of the corresponding areas obtained after euthanasia of the animal and graft explantation.

ISSUES

There are no major issues so far. However, it was necessary to restructure the partnership by adding a co-leader to Bioengineering (Dr. Kirby) and two co-investigators (Dr. Shiu in Bioengineering and Dr. Tasdizen in Imaging) to achieve our specific aims.

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PROJECT TITLE: Mechanical And Molecular Bases Of Endothelial Remodeling

PARTNERS' NAMES AND AFFILIATIONS:

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Dr. Jun-Lin Guan at Cornell University

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

ABSTRACT

The shear stress due to blood flow is borne primarily by endothelial cells (ECs) located at the interface between blood and vessel wall. Atherosclerotic lesions are preferentially localized in regions such as arterial branch points and local lumen expansions, where the ECs are subjected to disturbed flow conditions, including flow reattachment, low shear stress magnitude, high shear stress gradient, and little net direction of flow. The ECs in these regions have different structural and functional characteristics in comparison to those in the straight parts of the arterial tree, which are exposed to pulsatile flow with a large net forward direction. Our **Hypothesis** is that flows with a significant net direction cause adaptive changes in cell morphology to reduce surface stress and alter molecular signaling, such that the ECs can optimize their functions. In contrast, disturbed flows without a significant net direction do not elicit the same adaptive effects on EC surface stress distribution and lead to different spatial and temporal characteristics of molecular signaling, structural remodeling, and mechanical properties, thus resulting in distinct functional consequences such as vulnerability to atherosclerosis. The following five **Specific Aims** are proposed to test our hypothesis by using a combination of *in vitro* (first three Specific Aims), *ex vivo* and *in vivo* approaches: (1) To determine the effects of different shear flow patterns on surface stress and structural remodeling of ECs. (2) To elucidate the interplays between EC remodeling and molecular signaling in response to different shear flow patterns. (3) To elucidate the mechanisms by which different shear flow patterns regulate EC proliferation and apoptosis. (4) To establish the mechanisms by which different shear flow patterns regulate EC remodeling, molecular signaling and proliferation/apoptosis in blood vessels *ex vivo*. (5) To establish the mechanisms by which different shear flow patterns regulate EC remodeling, molecular signaling and proliferation/apoptosis in blood vessels *in vivo*. Dr. Shu Chien at UCSD, will lead the studies under all specific aims. Dr. Michael Sheetz at Columbia University will be responsible for identifying the roles of membrane tension and cytoskeleton affinity for cytoplasmic proteins in mechanotransduction. Dr. Jun-Lin Guan at Cornell University will study the molecular mechanisms regulating EC functions, especially in relation to KLF2 and FAK. The findings obtained from these studies will advance our fundamental knowledge on mechanotransduction and remodeling, thus providing the mechanistic basis for the development of novel approaches for diagnosis and treatment of cardiovascular disorders.

STATUS OF RESEARCH AND PARTNERSHIP

The traction forces exerted by a cell can be determined from the cell-induced displacements of fluorescent markers embedded in an elastic substrate. We have developed a method for measuring the 3-dimensional traction forces exerted by an adherent cell on the substrate (manuscript in preparation). Using this novel method, we have shown for the first time that a migrating endothelial cell exerts a significant normal traction at the cell-substrate interface, as well as tangential forces. The method we have developed will facilitate future studies on the biomechanical aspects of the proposed research on cell behaviors and functions

In addition to the traction forces generated by the cells at the cell-extracellular matrix adhesion sites, the forces at cell junctions can also play a role in regulating the force balance in the cell and cellular functions. We investigated the interactions between integrin engagement and cell junction VE-cadherin dynamics. Integrin engagement with beads coated with fibronectin (FN) led to the activation of Src (Src FRET reporter) around the beads and at adherence junctions, which are subsequently disrupted. These results indicate that integrin engagement disrupts VE-cadherin-containing adherence junctions via the activation of Src as a result of modulation of the actin network. Furthermore, we have also used this Src FRET reporter to monitor the Src signal map in response to local force application by applying laser-tweezers traction on FN-coated beads adhering to the cells. These findings demonstrated that the Src reporter we developed can be used to monitor mechanotransduction in live cells with spatial-temporal characterization.

In collaboration with our partners, we have made significance progress towards preparation and characterization of primary ECs from the floxed FAK mice for studies as outlined in the application. Optimal conditions are identified to delete FAK genes in the primary EC isolated from floxed FAK mice via infection with recombinant adenovirus encoding Cre (Ad-Cre). We found that inactivation of FAK in the isolated ECs caused increased apoptosis, reduced proliferation and migration, and reduced capillary formation on Matrigel. We also found that FAK deletion significantly reduced tyrosine phosphorylation of paxillin at Tyr118 and activation of JNK and Erk1/2. These results suggest that FAK plays an essential role in the regulation of multiple EC activities. The ability to manipulate FAK gene in isolated primary ECs also provided us with a good system to further dissect the roles of specific FAK downstream signaling pathways in the regulation of EC functions.

We have demonstrated that different flow patterns elicit distinct responses of Krüppel-like factor-2 (KLF2) in ECs in vitro and in vivo. While pulsatile flow induced sustained expression of KLF2 in cultured ECs, prolonged oscillatory flow suppressed KLF2 expression. The suppressive effect of oscillatory flow was Src-dependent. Immunohistochemical studies on ECs at arterial branch points revealed that KLF2 protein levels were related to local hemodynamics. Such flow-associated expression patterns were also demonstrated in a rat aortic restenosis model. Inhibition of KLF2 with siRNA sensitized ECs to oxidized LDL-induced apoptosis, indicating a protective role of KLF2. We also have successfully cloned the full-length human KLF2 cDNA and subcloned it into a Tet-off adenoviral vector for the over-expression of KLF2 (AdKLF2). Our results on a rat carotid artery stenosis model suggest that AdKLF2 is a potential agent that can be locally delivered to the ECs at the stenosis site to promote vascular remodeling. The mechanisms by which KLF2 regulates vascular remodeling *in vivo* will be further investigated.

ISSUES

There is no major issue for the scientific progress and partnership.

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PROJECT TITLE: Uropathogen Detection Using DNA Biosensors

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GRANTING NIH INSTITUTE: National Institute of Biomedical Imaging and Engineering (NIBIB)

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 the UroSensor system for the genotypic detection and species-specific identification of uropathogens. Engineering studies have resulted in development of a sample processing micro-fluidic system that enables concentration and lysis of uropathogens. 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 configurations of the DNA probes and in the assay kinetics. The lower limit of the sensitivity of the target derived from raw bacterial lysates without nucleic acid purification or amplification was $\leq 2 \times 10^4$ bacteria/ml. We have achieved the successful application of the 'UTI chip' to clinical urine specimens, with 100% sensitivity for identification of Gram-negative uropathogens. The optimal length of the probes, the distance between the capture and detector probes and the position of fluorescein modification were determined. For the first time, the optimal probe configuration allowed accurate detection within a time frame (30 minutes from sample collection to readout) and at room temperature, which would enable point-of-care diagnosis and treatment. The group also expanded this technology to successfully perform rapid (less than 2.5 hrs) molecular based antibiotic sensitivity detection. In addition, significant progress was achieved in implementation of a cartridge based micro-fluidic system.

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, biweekly laboratory meetings, and posting of electronic reports on our website.
Detection of Uropathogens in Clinical Specimens – Published Validation. 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. 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,*

Enterococococcus spp., *Klebsiella-Enterobacter* group and so on. 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. We have successfully applied the 'UTI chip' to clinical urine specimens. A clinical study was performed to compare electrochemical sensor assays with standard clinical microbiology results. We tested the analytic validity of the electrochemical sensor assays on 78 blinded clinical specimens. A preliminary algorithm for analysis of the sensor data was developed, indicating that the electrochemical sensor has 100% sensitivity for identification of Gram-negative uropathogens. This work has been published (*Liao JC, at al. J Clin Micro 2006, 44(2): 561-70*).

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. Studies were performed to determine the effects of probe length, fluorescein modification position, and distance between capture and detector probe hybridization sites. Improved signal intensity (>20-fold) were achieved by eliminating the gap between the capture- and detector-probe hybridization sites on 16S rRNA combined with 3'-fluorescein modification. Finally, the feasibility of a detector probe cocktail was demonstrated. This work has been submitted for publication and currently is under review.

Acceleration of Assay Kinetics and Operating Temperature. From the beginning of our project, we aimed at developing a rapid, low-cost and simple analytical technique which could be performed at ambient temperature at a variety of point-of-care situations. During the last year, we have made considerable progress in optimizing the assay procedure. By optimizing the probe configuration, the current use of shorter probes (less than 15mer) allow consistent and reliable analysis in less than 30 minutes at room temperature, thus eliminating the need and cost of a sophisticated temperature control system. 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.

Identification of Antibiotic Susceptibility. Antibiotic susceptibility studies are a separable companion of clinical laboratory species identification. Currently this procedure takes 48 to 72 hours to complete. That means, in most clinical situations, antibiotic selection is made by educated guess rather than evidence-based decision. We have found that the bacteria in optimal condition divide every 18 minutes and 16S rRNA electrical signal increase in parallel and exponential fashion. In the presence of sensitive antibiotics, no signal increase occurs. We have shown for the first time, our UroSensor, the molecular based technology, can give accurate antibiotic selection data within 2.5 hours. Preliminary clinical validation has been obtained by comparing the data from microbiology lab, which yielding the agreement rate of 91.3% (43 out of 47 comparison).

Microfluidics. GeneFluidics has successfully developed a cartridge based microfluidic system that is effective in semi-automatic molecular detection in bodily fluids from clinical patients. This cartridge system was composed of a reagent storage cartridge which could be developed for urine concentration and purification, bacterial lysis, reagent mixing and kinetic oscillation; and a sensor cartridge which could incorporate the current UroSensor and monitor the redox reaction. Oscillation in the system has been shown to increase the signal 3~4 fold. Our engineering collaborators are continuing to improve sample preparation. A system utilizing deionization and dielectrophoresic concentration of bacteria has been developed. These improvements could be integrated into the design requirement for the sample preparation system.

ISSUES

None.

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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 approximately 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 Advanced Research Institute (Virginia Tech) and from the University of Edinburgh (Scotland, UK). We will apply expression microarray and tissue microarray technologies and develop powerful new data analysis algorithms to define the gene expression profiles of up to 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 term, genes in this classifier 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 develop, 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 microarrays to validate and optimize the performance of these classifiers in a retrospective prognostic study of human breast tumors.

STATUS OF RESEARCH AND PARTNERSHIP

We continue to make substantial progress in our bioengineering studies to develop, validate, and share our algorithms with the broader community. In this regard, our partnership members have been highly active in the caBIG community and we are funded developers, adopters, and participants in multiple worskpaces. These activities are a direct extension of the bioengineering expertise and tools under development in Aims 2 and 3 of this BRP. For example, in the Integrative Cancer Research workspace, our **Vi**sual **S**tatistical **D**ata **A**nalyzer software (VISDA), as developed in this BRP, was selected as a key component of the gene expression microarray toolset for caBIG, and was supported by caBIG for development to silver level compliance. In the Architecture workspace, our team was recently funded to develop the caBIG Portal and we are an adopter for caArray, reflecting the expertise developed within our BRP to build our proposed data storage/sharing grid. We have established commercial collaborations to build caBIG tools related to clinical trials data management, also consistent with our BRP (Clinical Trials workspace). Development/extension of these tools as proposed within our BRP predates establishment of the caBIG community and its requirements for open source programming.

To explore fully high dimensional gene expression array data spaces, we continue to develop and test novel tools. We recently derived a stability analysis-guided supervised clustering and visualization method that can discover the hierarchical structure of phenotypes in gene expression data ("tree of phenotype"). To address the risk of overfitting, we embed a color-coded supervised-model VISDA (ccsmVISDA) learning procedure within a leave-one-out stability analysis to generate "leave-one-out trees". The final solution is the tree whose hierarchical class structure most frequently occurs, consistent with the maximum likelihood principle. This winning tree reflects the underlying stable structural information in the data because it is learned from the data set and survives small disturbances of the data set. Stability analysis-based ccsmVISDA algorithm (SAccsmVISDA) will lead to an improved generalization since, given different realizations of the data distribution, the algorithm will output similar solutions. We applied SAccsmVISDA to the MIT cancer data set (1); the frequency of the winning tree is 102/144 (0.71). We then built and tested a tree classifier, achieving a classification accuracy of 87.5%, comparing very favorably with that of Ramaswamy *et al.* (1) (a parallel multiple SVM model that provided a 78% classification accuracy). To explore the biological plausibility of our solution, we compared our tree to a tree based on pathologic and ontologic knowledge (2). Our solution has notable similarities to this tree, consistently classifying lymphoma, leukemia, CNS, and epithelial cancers into groups in which lymphoma and leukemia are closely related and CNS and epithelial cancers are closely positioned. Cancers of the uterus, breast, lung, colon, bladder, kidney, and pancreas also are appropriately classified.

ISSUES

Previously, our biggest problem had been accrual of sufficient numbers of informative cases. However, our colleagues at the University of Edinburgh recently provided an additional ~70 cases with adequate clinical follow-up.

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

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

ABSTRACT

The respiratory tract is the point of entry for a host of airborne materials that ultimately affect human health. Its complex structure and function, however, have traditionally confounded efforts to understand biological responses in humans based upon data derived in laboratory animals. To improve predictions of site-specific airway dose and response, this bioengineering research partnership (BRP) seeks to develop and test 3-dimensional (3D), biologically based models of the respiratory tracts in animals and humans. The overall specific aims are to: (1) develop and apply magnetic resonance imaging and fluorescent microsphere techniques to determine the dynamic, 3D structure and function of the living respiratory system; (2) determine its 3D cellular organization and metabolic capacity; (3) develop and extend computational capabilities for 3D modeling; (4) develop a normalized atlas of airway geometries with explicit measures of variability; (5) conduct *in vivo* gas exchange and particulate dosimetry studies for model validation; and (6) provide a web-based "respiratory physiome" for dissemination and training in the use of imaging and annotated model databases. Organizationally, work is divided into five projects, three technology cores, and an administrative interface.

STATUS OF RESEARCH AND PARTNERSHIP

In the second year of this BRP, methods developed in each of the three technology cores were effectively transitioned to support the five different projects. Highlights for each core and project are summarized below.

Small Animal Imaging Core: Work to optimize hyperpolarized ^{3}He gas production and delivery for magnetic resonance imaging (MRI) of respiratory structure and function in ventilated rats was completed. The gas production facility was engineered and built by Dr. Brian Saam's research team at the University of Utah, and performance was optimized and quantified at PNNL by Dr. Rick Jacob. Recent work by Dr. Jacob has focused on doubling overall gas production to better support requirements for *in vivo* studies. For live animal imaging, Dr. Minard fabricated dual tuned radio-frequency probes for 3D ^{1}H and ^{3}He imaging. These probes are now used routinely to comprehensively describe anatomical structure, airway and tissue mechanics, and airflows during controlled breathing in ventilated rats.

Computational Core: Drs. Dan Einstein, Andrew Kuprat, and Senthil Kabilan continue to refine techniques for airway segmentation, scale-invariant meshing, and CFD simulations for transient flows at PNNL. Dr. John Fowler at CGC, and Dan Einstein, (PNNL) have teamed to develop new approaches for generating, editing and labeling voxel-based medial axis's of rat pulmonary airways for subsequent statistical analyses and normative geometry atlas development (a.k.a. RAtlas).

The Physiome Core: Dr. James Bassingthwaighte and colleagues at UW have developed or implemented numerous published models of gas exchange that will be compared with experimental data. Eighteen of the models are now available on the physiome website (www.physiome.org/models). One of the long range goals is to begin to develop linkages between the (1D and 2D) gas exchange and particle dosimetry models, cellular and tissue properties, and airflow solutions generated from (3D and 4D) CFD models run on supercomputers. The result will therefore enable meaningful simulations of highly complex relationships that can be run on standard laptop computers. Dr. Gary Christensen and colleagues at Iowa have further developed their interface for creating the digital RAtlas and have begun incorporating data from lung casts imaged at PNNL. Once the normative airway geometry atlas is developed, future projects will link the cellular structure/function information with their appropriate airway architecture.

Project 1: Dynamic Structure and Function: Drs. Minard (PNNL) and Robertson (UW) completed their evaluation of imaging the distribution of combined fluorescent/magnetic particles in the 40 nm to 10 μm range. They have also begun studies to compare MR imaging of particle deposition in the lungs of rats with measurements made by cryomicrotome/fluorescent detection. Using the cyromicrotome method, acinar regions, which are below the resolution of the MRI, can be detected. By injecting a particle with a different fluorescent label intravenously, the heterogeneous distribution of ventilation/perfusion can be determined in the same animal. However, methods for extracting conducting airways from cryomicrotome images are needed to enable co-registration of particulate deposition in airways identified by MRI. Co-registration techniques are currently being evaluated by Drs. John Fowler, CGC, James Carson, PNNL, and Julie Kimbell, CIIT-CHR.

Project 2: Airflow & Mechanics: Drs. Minard and Jacob (PNNL) developed MRI techniques for quantifying airflow dynamics, visualizing airway architecture, and measuring regional tissue mechanics in live rats. Dr. Laicher (Utah) developed advanced 3D data reconstructions algorithms to facilitate 3D ^{3}He imaging. To compensate for the blurring of measured ^{3}He flow lamina that is caused by rapid gas diffusion during MRI data acquisition, mathematical relationships developed by Dr. Minard were applied to the computational fluid dynamics (CFD) simulations conducted by Dr. Dan Einstein and Senthil Kabilan, PNNL. The resulting CFD models were thus able to accurately predict the gas flow experiments conducted in model airways the size of the rat trachea. Experiments are in progress to match CFD simulations and MR measurements of ^{3}He gas flow in more complex geometries such as a rat nasal airway model and during live animal imaging.

Project 3: Cell Structure & Function: Drs. Plopper and Buckpitt, UC Davis, developed statistical sampling methods for defining cell populations in pulmonary airways by immunohistochemistry and genomic/proteomics methods for defining proteins involved in oxidant protection and chemical metabolism.

Project 4: Gas/Vapor Dosimetry: Dr. Hlastala and colleagues at UW have refined their methods for measuring gas or vapor exchange in rats using gases of varying water solubility. Studies are currently underway to provide data for validation of the 1D and 2D gas exchange models from the physiome core.

Project 5: Particulate Dosimetry: Drs. Timchalk, Minard et al. at PNNL, have begun live animal particle deposition studies using intra-nasal aerosolization methods in preparation for full inhalation exposures to fluorescent/magnetic microsphere studies that are planned in rats (and mice) in years 3 to 5 using the nose-only exposure system recently constructed at PNNL. Comparisons between *in vivo* MRI results and data acquired with fluorescent microspheres using the cryomicrotome will be critical for enabling non-invasive imaging protocols not only for visualizing structure-function relationships but also for validating computational models of particulate dosimetry carried out by Drs. Timchalk et al. at PNNL and Asgharian at CIIT-CHR. Dr. Marshall Richmond and Bill Perkins, PNNL, have begun development of an open-source Lagrangian particle tracking code that can be coupled with CFD simulations for evaluating deposition in dynamic flows with moving boundaries.

ISSUES

None.

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PROJECT TITLE: Spatiotemporal Brain Imaging: Microscopic & Systems Level

PARTNERS' NAMES AND AFFILIATIONS:

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

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). During the past two years (year 3 and 4), we have applied these newly developed technological tools to the study of stroke animal models (Aim4).

STATUS OF RESEARCH AND PARTNERSHIP

Aim1: improve fMRI spatial resolution. We have developed a 4-channel phased array coil for non-human primate imaging at 7T and routinely acquire fMRI data with awake behaving macaques at a resolution of 0.75mm isotropic. The 4-channel phased array coil consists of four coils of five centimeters in diameter arranged perpendicular to the axial plane with a left-right symmetry around the head. We have achieved a four-fold increase in SNR over a conventional single 10cm coil and achieve image SNR values of 60 and temporal SNR values of 40 at a resolution of 0.75mm isotropic.

Data are acquired using a customized segmented GRE-EPI sequence, which allows for accelerated acquisition of half of the k-space lines resulting in a strong reduction of the EPI image distortions. We further developed MATLAB code for SENSE reconstruction and motion correction of the images. Sensitivity profiles are created based on T1 weighted images acquired with a FLASH sequence. Alternating acquisition of the odd and even lines in k-space allows for a reduction of noise in the time domain.

Image stability has improved due to the fast data acquisition with an acceleration factor of two and use of the fast 80/800 gradient system of the 7T scanner. In addition we use the axial plane (x-z in scanner) as the imaging plane, which results in minimal distortions in the images and subject motion mainly leads to shifts of the images due to B0 changes but no further distortions in the images. Applying a slice-by-slice motion correction allows for precise correction of these shifts and typically less than 5% of all images are excluded from analysis because of misalignment or increased image distortion. Consequently, the subjects do not need to be restraint or trained to avoid body motion during the scans.

Aim2: improve the spatiotemporal resolution of optical imaging. Multi-photon microscopy is a well established technique. Only in the past few years however has in-vivo two-photon microscopy been

demonstrated. The major benefit of using multi-photon microscopy over conventional histological examination of tissues is the ability to image non-destructively to depths of up to 500 μm. In addition, numerous new dyes, targeted probes and transgenic mutants have become available to allow in-vivo imaging of specific physiological and biochemical processes. Two-photon microscopy offers improved depth penetration and reduced photo-damage compared to confocal microscopy, making it particularly suitable for in-vivo imaging. We have developed an optimized custom-built in-vivo two-photon microscopy system capable of video-rate (>22 frames per second) high-resolution imaging to depths of > 450 microns. The two photomultiplier tube detectors allow spectrally distinct fluorophores to be imaged simultaneously. The spectral choice can readily be altered by interchanging filters, and additional PMTs can be added where necessary. Our Mai-Tai laser system from Spectra Physics provides high power tunable from 710nm to 920nm, allowing excitation of Dextran-Conjugated Fluorescein, Oregon Green 488, Texas Dextran Red and Green Fluorescent Protein among others.

Aim 3: apply the new technology to image functional activation. We have used the two-photon system (see Aim 2) to image in vivo vascular responses in identified single vessels in response to forepaw stimulation in healthy rats and have combined two-photon imaging of vascular responses with calcium imaging (using calcium-sensitive dye Oregon Green BAPTA-1 AM). Specifically, we have addressed the question of neurovascular coupling on the level of single blood vessels (arterioles and venules). Prior to two-photon imaging we performed electrophysiological mapping using a "ball" electrode in order to determine the center of electrophysiological activity in response to a somatosensory stimulus (1sec-train of electrical pulses, 300 μs at 3Hz, 1mA). Our data show that all surface arteries/arterioles (diameter of 10-100μm) within 1.5mm of the center of neuronal activity dilated (up to 20%) with an increase in velocity up to 30%. The response onset and amplitude correlated well with distance from the center of neuronal activity. In addition, many arterioles showed a vasoconstriction that followed the initial dilation (see figure). This vasoconstriction was in particular evident in remote locations relative to the center of neuronal activity where a small initial dilation was observed. There was no measurable diameter change on the venous side. However, venules showed velocity increases up to ~ 30%.

Aim 4: apply new technology in established neuropathological models. fMRI is beginning to make inroads into clinical applications including monitoring of stroke rehabilitation. However, the functional significance of plastic changes revealed by fMRI is poorly understood, largely due to the lack of understanding of the interplay of neuronal and cerebrovascular mechanisms under this clinical condition. Here we apply optical imaging, electrophysiology and immunohistochemistry aiming for mechanistic interpretation of non-invasive imaging data.

We examined cortical responses to a somatosensory stimulus two weeks after transient (2hr) unilateral occlusion of middle cerebral artery. The stimulus consisted of 2sec-train of weak electrical pulses delivered to either right or left forepaw. Neuronal activity was recorded using laminar electrode arrays and also using voltage-sensitive dyes (VSD). Hemodynamic activity was measured using spectroscopic imaging of blood oxygenation and speckle imaging of blood flow.

In comparison with stimulation of unaffected limb, stimulation of affected limb featured: (1) reduced amplitude of hemodynamic and VSD signals in contralateral SI; (2) spatial shift of the active contralateral region in the posterior direction; (3) bilateral VSD signals of comparable amplitude and post-stimulus delay (upon stimulation of the unaffected limb VSD response in the ipsilateral SI had a longer delay due to signal traveling via corpus callosum). In the majority of cases the ipsilateral VSD signals were unaccompanied by any detectable hemodynamic response.

Electrophysiological recordings showed an abnormal shape of the local field potentials on the affected side indicative of a change in neural circuit dynamics. Multiple unit activity recorded from the affected hemisphere showed that an increase in neuronal firing was lacking a subsequent decrease characteristic of control conditions. These findings suggest a possible reduction in number of inhibitory interneurons.

Immunohistochemical labeling for specific classes of inhibitory interneurons (expressing NPY, SOM, VIP and NOS) are underway in our laboratory to address this hypothesis.

ISSUES

None.

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PROJECT TITLE: Proteomics to Biomimetic Polymers: Engineering Proteins for Antimicrobials

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB), first year by National Institute of General Medical Sciences (NIGMS)

ABSTRACT:
Purpose: Our understanding of the structural basis for protein function is rapidly evolving as a result of modern approaches to structural proteinomics. Our intention is to use this understanding as a starting point for the design of biomimetic polymers that are much more stable and inexpensive to produce than natural proteins, but nevertheless mimic their key biological properties. A primary goal of this project will be to design polymer and defined-length oligomers capable of presenting functional groups in arrays similar to those found in natural, biologically active proteins. To illustrate this approach, we will design mimics of a class of membrane-active antimicrobial peptides and proteins. A large class of antimicrobial peptides adopt positively charged amphiphilic alpha-helices, in which changed polar groups and apolar groups line up on opposite faces of the helical cylinder. Here, we propose to develop computational methods to aid in the design and analysis of a variety of other antimicrobial polymers or oligomers that are simpler in structure and hence much less expensive to produce than either alpha- or beta- peptides. The antimicrobial activities of these polymers will be tested in solutions and as part of solids surfaces. Their structures and mechanisms of action of the polymer and oligomers will be evaluated using a battery of biophysical methods, as well as by using gene chips to examine which genes are turned on by sub-lethal concentrations of the compounds.
Methods:

MIC- These experiments follow standard NCCLS protocols for micro-broth dilution assays adapted to 96-well plates. The plates are allowed to incubate for 6 or 20 h at 37°C before the OD600 is measured.

Hemolysis – Following standard procedures and measuring release of hemoglobin spectrophotometrically at 414nm. Triton-X is taken as 100% lysis.

Results: In the final year of the granting period we have focused considerable effort on design of not only polymers but also small molecules mimics that address problems with host defense peptides. We have developed short folded arylamide oligomers that mimic the structures and activity of antimicrobial peptides. In the most recent period we fine-tuned the conformational properties of these foldamers to improve their antimicrobial activity and selectivity. Alkoxy groups were introduced into the arylamide template to rigidify the conformation *via* hydrogen bond formation, resulting in increased activity towards *Staphylococcus aureus* and *Escherichia coli*. Simultaneously, these changes decreased toxicity towards mammalian cells. Distinct structure-activity relationships emerged from rigid versus more conformationally mobile series of foldamers. A membrane potential-sensitive dye 3,5'-dipropylthiacarbocyanine iodide ($DiSC_3(5)$) was used to investigate the interaction between amphiphilic cationic arylamide foldamers and the cytoplasmic membrane of *Staphylococcus aureus*. The compounds induced very rapid loss of the membrane potential, although complete loss of the potential was not observed at the minimal inhibitory concentration. We also examined the development of resistance by culturing *Staphylococcus aureus* at concentrations of the antibiotics just below their minimal inhibitory concentrations. Resistance to conventional antibiotics, ciprofloxacin and norfloxacin, was rapidly developed but no change of antimicrobial activity of the arylamide foldamers was observed over a course of sixteen passages. Importantly, in the mouse thigh burden experiment, our compound reduced bacterial burden at doses (5 to 10 mg/kg, *i.v.*) similar somewhat lower than the peptide antibiotic, vancomycin (10 mg/kg, *sc*.). These results demonstrate the potential of arylamide foldamers as systemic agents for the treatment of antibiotic-resistant bacterial infections.

Conclusions: The design principles enable many different structures to be prepared with potent and broad spectrum activity while maintaining limited toxicity to mammalian cells. Combining the power of synthetic and computational methods with overarching bioengineering principles created a powerful approach to infectious disease treatment.

STATUS OF RESEARCH AND PARTNERSHIP

Beyond these MIC and HC_{50} experiments, membrane studies, *in vivo* experiments, and computational designs are progressing. The number of students and post doctoral fellows involved in the program continues to increase. Collaborations beyond the partnerships are growing with several joint publications in press or currently being written.

Excellent scientific progress has been made. Many publications are in the literature or in press. A start-up company has been created that has already raised ~$8 million dollars and has six employees. The PI's continue to collaborate strongly.

ISSUES

None

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PROJECT TITLE: Image Guided Intracardiac Beating Heart Surgery

PARTNERS' NAMES AND AFFILIATIONS:

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Gerald Marx MD, John Triedman MD, Dept. Cardiology, Children's Hosp., Boston, MA,

GRANTING NIH INSTITUTE/CENTER: National Heart Lung and Blood Institute (NHLBI)

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

Throughout our project, we have taken a two pronged approach to development of ultrasound guided intracardiac surgery. The first is to improve current 3D ultrasound imaging to provide realistic "surgeon's orientation" imaging of Intracardiac structures an instrumentation to enable accurate and reproducible tissue reconstruction and device deployment. The second has been to develop the surgical tools and devices specifically designed for per cardiac repair of intracardiac defects.

The challenges of the first component, ***3D ultrasound imaging***, have been the limited resolution of tissue structures, imaging artifacts generated by surgical instrumentation, limited field of view for instrument navigation, need for accurate tissue and cardiac structure segmentation and tracking throughout the cardiac cycle. In our initial work we were able to identify methods of optimizing the current US system for image guided surgery by calibrating gain settings, standardizing probe position, identifying optimal probe to instrument angle, and developing wide angle field of view. All of these developments aid navigation and instrument visualization. However, to improve resolution and minimize artifact, more complex solutions are required. The most significant advance that has enabled complex solutions to these problems has been our development of a streaming data port of pre-volume rendered data from the Sonos 7500. Real-time image processing, stereoscopic displays, image based instrument segmentation and tracking, and tissue feature tracking in 3D have now been developed with the close collaboration between engineering groups from university and corporate laboratories, and clinical researchers. Preliminary work has begun on development of automated instruments that use tissue tracking in 3D to control instrument motion and target tissue structures for device deployment. Experiments are also planned using higher frequency ultrasound probes, optimized for near field imaging to provide higher resolution imaging still keeping the post-acquisition processing capabilities available with the current system.

For the second part of the project, ***instrument development***, we have developed methods for surface modification of surgical tools and devices to limit imaging artifact and optimize visualization with current imaging probes. Devices for closure of atrial septal defects (ASD) and repair of mitral valve regurgitation are being developed, specifically for ultrasound guided surgery (patents pending). Further development of the devices for ASD closure for use in humans is currently being done by a manufacturing firm under contract with the P.I. and Children's Hospital-Boston.

ISSUES

The most significant issue encountered in the first year of the BRP was finalizing the sub-contract and Intellectual Property agreement between the academic institutions involved in the BRP and Philips Medical Systems. Many issues regarding IP, prior to and as a result of the BRP activities, had to be resolved. This delayed the development of the data streaming interface to access the datastream from the Philips ultrasound equipment for off-line processing. This issue has now been resolved.

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PROJECT TITLE: Proteomics of Cell Death Via 2-D Microfluidic Profiling

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GRANTING NIH INSTITUTE/CENTER: NIGMS

ABSTRACT

Current proteomic studies are largely based upon two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) due to its ability to provide detailed differential views of thousands of proteins expressed by an organism or cell type under varying physiological states, while also providing an excellent platform for evaluating post-translational protein modifications. However, 2-D PAGE suffers from a number of constraints, including poor throughput and high sample loading requirements, which make it poorly suited to broad-scale biomarker discovery from human clinical specimens. This BRP project addresses these limitations through the development and demonstration of a suite of innovative microfluidic technology platforms based on multidimensional protein separations combined with MALDI-MS for protein identification and quantification. One of the primary separation platforms under development combines isoelectric focusing (IEF) and sodium dodecyl sulfate (SDS) gel electrophoresis on a single microfluidic chip. The overall research effort combines three interrelated tasks. The first task is focused on fundamental microfluidic platform development, including fabrication, modeling, imaging, and optimization of multidimensional protein separation platforms capable of providing a peak capacity of at least 10,000, measurements of pI and MW to within 5% of theoretical values, and an analysis cycle time of less than 10 min under manual operation. Validation will be performed during the second year of the project using *E. coli* cell lysate as a model system. The multidimensional microfluidic protein profiling technology will next be integrated with MS analysis, enabling direct and rapid biomarker identification. The interface will be realized by transferring proteins from multiple parallel on-chip separations to a MALDI target using a newly-developed microfluidic electrospray deposition method, followed by MALDI-MS protein identification. An alternate approach will leverage a newly-developed multidimensional protein separation platform which combines isoelectric focusing with nanoscale reversed-phase liquid chromatography and MALDI-MS. Ultimately, automation of the microfluidic profiling platform will be performed, with a goal of realizing a "push-button" system which will perform sample loading, multidimensional separations, and protein pattern image capture from a disposable plastic chip, followed by 2-D image analysis and differential display between multiple chips. Further enhancements in reproducibility and throughput of protein profiling together with significant reduction in sample requirement will be realized through automation.

STATUS OF RESEARCH AND PARTNERSHIP

During this first year of the research effort, the key fabrication challenges for the microfluidic system have been addressed. Using UV-transparent polymethylmethacrylate (PMMA) substrates, a variety of designs have been fabricated by hot embossing from deep reactive-ion etched (DRIE) silicon wafers. Embossing and thermal bonding conditions have been optimized to achieve consistent results with less than 5% variation in channel width and height across a full 8 cm diameter area. A new UV-ozone surface treatment has been developed to produce highly hydrophilic PMMA surfaces for improved liquid filling

and reduced biofouling, and to improve the bond strength of the microfluidic chips without resorting to high bonding temperature which could distort the microchannel geometry.

An enabling technology for reducing one of the most critical challenges facing the effective implementation of complex, multiplexed microfluidic systems has been validated. Historically, microfluidic systems containing parallel coupled channels have been demonstrated with only limited success due to problems with electrical and hydrodynamic crosstalk between the coupled microchannels. In addition, as the number of reservoirs increases due to the more complex nature of the multidimensional microfluidic system, challenges associated with fluid leakage and unbalanced fluid flow become increasingly challenging. To avoid these problems, porous polyethyleneglycol (PEG) gel plugs fabricated by in-situ UV-initiated polymerization have been implemented to control electrical and fluidic resistance within selected branches of the microfluidic system. A surface silanization chemistry has also been optimized to produce covalent wall attachment with the PEG gel. This approach has been shown to successfully eliminate unwanted bulk flow of sample within the chips to ensure consistent performance during sample injection and separation.

Further developments during the first project year include demonstration of PEG gel formulations suitable for high-efficiency microfluidic protein separations, high-efficiency IEF separations within multichannel devices, and >4x sample stacking during transfer from the IEF separation dimension to the CGE dimension simultaneously across multiple 2^{nd} dimension channels. In addition, a new approach to spotting sample from multiplexed microfluidic chips to prestructured MALDI targets containing hydrophobic virtual sample wells has been validated, and is currently being scaled up for compatibility with a chip containing 128 parallel 2^{nd} dimension microchannels.

The partnership combines key skillsets in advanced microfluidic system fabrication, analytical chemistry, and system engineering, each of which is essential to success of this BRP project. By leveraging the combined capabilities of the research team, all deliverables for the first project year have been completed ahead of schedule. Primary goals for the coming year include full demonstration of the microfluidic separation platform using *E. coli* cell lysate as a model system, combined with MALDI-MS interfacing with sub-fmol detection sensitivity for a range of peptides.

ISSUES

No issues to report at this time.

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PROJECT TITLE: A 3-D Microfluidic/electronic Neural Interface System: In Vitro Studies of Neural Networks, Plasticity, and Injury

PARTNERS' NAMES AND AFFILIATIONS:

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institute of Neurological Disorders and Stroke (NINDS)

ABSTRACT

The focus of the research program is to advance the knowledge of the functionality of neural circuits and networks through the development of a set of technologies that facilitate the study of three-dimensional (3-D) neural cell and tissue cultures. We are creating a microfabricated neural interface system (μNIS) by combining an array of micromachined towers that incorporate microelectrodes and microfluidic channels. These towers are fabricated on a substrate that will process the signals to and from the towers using integrated circuits. In addition, the towers will have microfluidic channels through which flow of nutrients or other substances is highly controlled. The resulting system will enable a new field of neurobiological research, in which the collective properties of 3-D neural circuits can be observed and manipulated with unprecedented control and precision, in response to both normal conditions and traumatic disruption of the circuits.

As described in the original proposal the project has four specific aims, each of which contains a set of technological developments motivated by biological hypotheses:

Specific Aim 1: Fabricate arrays of 3-D microtowers that will support neuronal cell growth and permit integration with microelectrode and microfluidic structures.

Specific Aim 2: Develop a novel multi-site, three-dimensional microfluidic system to locally control the delivery of neural stimuli/nutrients in order to improve cell survival, deliver chemical stimuli, and examine growth and network formation.

Specific Aim 3: Develop custom integrated circuits that will incorporate amplification, multiplexing, and processing of the neuronal data, and will facilitate simultaneous stimulation and recording.

Specific Aim 4: Combine technologies developed in Aims 1-3 (microfabricated towers, microfluidics, and electrical interfacing) with 3-D neural tissue to study information processing, learning, and the morphological and network response to traumatic injury.

The study of nervous systems has revealed that global system behavior is a reflection of the cellular and molecular behavior at the individual neuron level and the electrical behavior at the network level. Neural networks in the intact brain are highly complex electrochemical control systems and the ability to probe and image cultures with improved spatial resolution will enable advances that are otherwise impossible to attain. The significance of this research lies in the ability to create robust neuronal networks in an in vivo-like cytoarchitecture and precisely study neuronal behavior in normal and injured tissue. Technologies developed for this research will greatly expand the range of possible electrical and chemical neural studies. Studies resulting from this research will lead to greatly enhanced in vitro investigations and provide technology for the development of "smart" neural implants for replacement of lost sensory and motor function in humans.

Status of Research and Partnership:
The results to date from Year 4 have proceeded in each of the Aims as follows. Additional information regarding progress on the project can be found at http://www.neuro.gatech.edu/brp/

Specific Aim 1: We have expanded the range of available tower fabrication technologies with a view towards batch fabrication and with smaller feature sizes to explore their use in brain slice research. The Allen group has fabricated and validated the previous design, and has provided versions of these for further biological validation. However this design uses a serial process for electrode and conductive trace construction, which makes it problematic for large electrode counts. This shortcoming has prompted the development of new technologies based on metal transfer micromolding, which allows for much simpler and faster fabrication steps with additional flexibility towards design changes. Test devices using this technology are currently in the final stages of fabrication and designs specific for slice work are being elaborated. The Brewer group has been using the tower design provided by the Frazier group to validate the use of continuous perfusion on cultures in these towers. It should be pointed out that, without an external matrix, cells will only grow on the surfaces of the tower and the bottom substrate in what we refer to as a 21/2D culture. The LaPlaca group has been using towers provided by the Allen group to study perfusion on a full 3D culture using matrigel as a cell support structure away from the towers, the same principles have been transfered to the Wheeler group where it is being adapted to the creation of differentiated 3-D neural cultures (cell bodies vs. cell processes). As a look towards more economical processing, the Wheeler group has been evaluating microstencils for the fabrication of partially disposable planar multielectrode arrays.

Specific Aim 2: The Glezer group has developed a variety of chambers to provide fluidic functionality to the designs provided by the Frazier and Allen groups and used by the Potter, LaPlaca, and Brewer groups. These designs take into consideration evenness of perfusion, nutrient delivery, waste removal, imaging constraints, gas exchange, asepsis, and temperature regulation thus extending long term viability of both 3-D neural cultures and brain slices. The LaPlaca and Potter groups, in collaboration with the Glezer group, have evaluated planar and 3-D culture perfusion, showing improved neural viability in perfused cultures with close to 90% viability in 3D cultures at 20 DIV. The Brewer group has also shown improved viability in 2.5-D cultures with respect to the unperfused controls however in this case the 14-DIV viability is below that of a control slip. New tower designs by the Frazier group in collaboration with the Glezer group are being evaluated for the effects of multi-level perfusion in brain slices.

Specific Aim 3: The DeWeerth group, in collaboration with the Wheeler and Potter groups, has developed an integrated circuit that is capable of near–simultaneous stimulation and recording from neural cultures surpassing any existing commercial system, and on doing this has expanded the understanding of stimulation artifacts and its elimination, and the range of experiments that can be achieved by modifying stimulation parameters. Using the resulting system the Wheeler group has shown neural recordings as soon as 4ms after stimulation and 1.2ms in adjacent channels. Given that the artifact is eliminated at the electrode itself, traces recorded from an MCS system in parallel with ours show similar behavior. This last property allows for protocols with long stimulation sequences, avoiding the “dish saturation” phenomena and enabling experiments that where not possible in the past. Our current efforts in this aim are in further reducing the artifact discharge duration, and on expanding the ICs to 64 or more electrodes.

Specific Aim 4: The Potter group continues the development of imaging techniques collaborating with the Glezer group in the development of devices to fit the existing environmental and imaging chambers. Additionally calcium imaging is being developed to study the range of stimulation of electrodes in our 3-D arrays (Potter and LaPlaca groups) and to allow for the development of stimulation protocols for selective stimulation of neurons in a dense culture (DeWeerth group). The design of these chambers takes into account electrical connectivity, and using the information from previous iterations, and simplifications of device construction, are being targeted to both neural culture and brain slice work. The Glezer group has created the first packages to incorporate both fluidic and electrical functionality packaging the first generation tower array design from the Allen group. The Wheeler group is pursuing generation of structured cultures with clearly differentiated processes.

ISSUES:

Although we are collaborating across three universities and five separate departments, our interactions seem to be effective. We have monthly video conferences of the entire team (faculty and students) and other video conferences of subgroups as needed. We also have semi-annual two-day retreats in Atlanta. The primary challenges that we have faced as a team is developing a shared understanding of the effort that it takes to design and implement technology, to validate that technology with biological tissue, and to apply that technology as a tool for studying the nervous system. The members of the team have formed subgroups that addressed specific technologies and applications successfully.

The primary technical challenges that we have faced have centered around the integration of the disparate technologies and their application to biological problems. Our project is very integrated in the fact that the "critical paths" of our subgroups are very intertwined. Although our communication has been effective (as described above), this level of integration still provides a daunting challenge. In the past year, we have made substantial progress on integrating technologies and applying them to biological applications, and ultimately toward reaching the overall goals of the project.

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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 (NIGMS)

ABSTRACT

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.

STATUS OF RESEARCH AND PARTNERSHIP

Our BRP is focused on developing the tools needed to enable solid-phase and array-based biocatalytic lead synthesis and optimization in high-throughput. This includes applied studies of enzymatic derivatization of leads bound to solid surfaces (beads or microarray slides), as well as fundamental studies of proteins solubilized in organic media and elucidating the structural features of enzyme selectivity in different media using a bioinformatics strategy. 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 - RPI**)[1]. We have overcome the poor accessibility of enzyme molecules into organic resins by using controlled-pore glass (CPG) beads with a pore size of 550Å. Moreover, selective cleavage of substrate and products from amino-functionalized CPG was performed using α-chymotrypsin under mild conditions. As a result, we have now demonstrated solid-phase peroxidase-catalyzed phenolic coupling, chloroperoxidase-catalyzed aromatic halogenation, halohydration, and epoxidation, and tyrosinase-catalyzed phenolic hydroxylation reactions. We are now extending the work to glycosidases (sugar addition) and dehydrogenases (oxidation and reduction) to round out a series of synthetically relevant biotransformations. In addition to CPG, we have also performed peroxidase catalysis on functionalized glass microarrays. Phenolic seed molecules were attached to glass slides and soluble peroxidase was added in the presence of different phenols. Arrays of phenolic coupled products were generated. These phenolic libraries may be useful in screens involving the inhibition of SH3-proline rich protein assemblies in cell signaling cascades [2].

Another area of work involves the development of selective attachment and removal strategies for complex leads. **Clark (UC Berkeley)** has made progress in attaching and subsequently cleaving one of our key leads, the flavonoid bergenin, onto carboxylic acid derivatized controlled pore glass (CPG-COOH, 500Å pores) with >95% yields. The cleavage in this system has been performed in organic solvents using lipases dissolved in the organic milieu. **Clark and Dordick** have also investigated the unique solubilization procedure to enable enzymes to function in soluble form in nonaqueous media. are currently investigating the use of these enzymes in both the soluble and native forms for carrying out a range of transformations in both organic and aqueous systems. Referred to as direct solubilization (DS), this method yielded solubilization efficiencies for subtilisin Carlsberg (SC) of up to 72 ± 3%, nearly 3-fold higher than optimized extraction efficiencies (25 ± 5%) with the same enzyme[3]. **Davison and Borole (ORNL)** has focused on PEG-modification of enzymes, which in the presence of surfactants to solubilize enzymes results in a 4-8 fold improvement in catalytic activity.

Nature owes its unparalleled structural and functional chemical diversity to the power of multi-enzyme pathways that comprise the synthetic machinery of biological systems. Mankind has only been able to tap into a small part of this biocatalytic repertoire, yet this has resulted in a vast array of natural products for use as pharmaceuticals and raw materials for chemical intermediates and polymeric materials. Nevertheless, a significantly more diverse of natural compounds remains untapped because of the difficulties of compound screening, production, purification and identification. We have used solution-phase enzyme reactions on microarray-bound polyketide synthase (PKS) precursor substrates to generate libraries of polyketides. Specifically, type III PKS and post-PKS tailoring enzymes (e.g., peroxidases, chloroperoxidases, and tyrosinases) to generate a series of pyrone and flavonol derivatives on a chip. These polyketide analogs were then evaluated as the tyrosine kinase inhibitors[4], attractive targets in the development of new cancer drugs in high-throughput.

Klibanov (MIT) has designed a simple natural algorithim that produces native active site sequences of enzymes using the sequence of the *Streptomyces* R61 DD-peptidase and those of two enzymes transforming primary biological substrates, namely nucleotides (thymidylate synthase) and sugars (β-galactosidase). Whereas simple optimization of binding affinity reproduced the sequence of the peptidase active site with high precision, imposition of additional, geometric constraints on side-chain conformations based on the catalytic mechanism was required with the other two enzymes. With such modifications, the sequence optimization algorithm correctly predicted 78% of the residues for all of the enzymes, with 83% being similar to native (and 90% correct, with 95% similar, excluding residues with high variability in multiple sequence alignment). In addition, the enantioselectivity of horseradish peroxidase (HRP), was improved by developing an experimental platform based on yeast surface display and FACS technology. Two libraries of HRP variants were constructed, each containing ca. $2x10^6$ unique sequences using error-prone PCR or by replacing 5 residues near the active site with the 19 non-Cys residues. The screening of the latter library afforded up to an 8-fold greater enantioselectivity, including its reversal, compared to the wild-type. In contrast, the library constructed using error-prone PCR yielded no variants with significantly altered enantioselectivity. Thus mutations close to the enzyme active site seem to impact the enantioselectivity far more than the distant ones[5].

ISSUES

The Partnership has been running relatively smoothly.

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PROJECT TITLE: Rapid Flow Evaluation by Magnetic Resonance Imaging

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GRANTING NIH INSTITUTE/CENTER: NHLBI

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 valvular diseases. Currently, valvular 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 represent major flow characteristics in a manner similar to conventional scanning (taking longer to acquire). CFD calculations have shown that adequacy of temporal MRI flow data is the dominant factor affecting accuracy when studying pulsatile flow. BRISK allows improved temporal resolution to be achieved when representing pulsatile flow. 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. Further, by explicitly incorporating knowledge of the temporal distribution of data, we were able to devise an algorithm to temporally align 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.

The focus of the adequacy of temporal resolution and temporal distribution of VEC data was not considered without due attention to spatial resolution which is required to represent convective acceleration (i.e. acceleration in velocity that takes place over voxels, even in the absence of temporal changes). We have shown that under-representation of either the temporal or spatial resolution introduces additional sources of error in representing flow-fields with high acceleration terms. We performed systematic investigation of these parameters using CFD based simulations in widely different physiologic flow conditions, including jet flow into a chamber, pulsatile arterial flow, and helical pulsatile flow through the aortic arch. We demonstrated that each flow-field condition required different degrees of spatial and temporal resolution to accurately represent the flow-field. In each flow situation, however, accuracy was usually dominated by one parameter over the other. For example, in jet flow imaging, the dominant requirement is that high temporal resolution be achieved.

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. Further, with the use of CFD modeling we are gaining additional insights into the error sources inherent in MRI VEC imaging. Thus, our standard of reference for velocity flow-fields is the CFD model, and not the conventional VEC data. This increased understanding is a direct result of the cross-disciplinary nature of the investigation. We conducted two studies directly comparing VEC and Doppler echocardiography to show that when comparing VEC to echocardiography, that errors in VEC imaging predicted by CFD modeling were detectable in patients. Thus, we are now focused on accuracy of FRISK VEC as opposed to reproducing the results of conventional VEC. In summary, we are very encouraged by the partnership and believe that its very structure has contributed to success of 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 (NIBIB) and National Institute for Neurological Diseases and Stroke (NINDS)

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 academia and industry 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 as follows:

1. Coil Development for high resolution MRI/MRS: Work in the area of developing a dynamic shimming strategy to improve MRS acquisitions continued. As described in the previous report, we have developed a volume coil-CRC coil phased array for simultaneous reception, providing up to 35%

increase from central brain regions. We have extended this work to produce a circumscribing, counter-rotating surface coil (CRC) array yielding improvements through out the entire brain.

2. MRS- Biochemical imaging of Epilepsy: We have continued to develop a novel method for adjusting B0 inhomogeneity in difficult brain regions. Also, recently we have developed a moderate echo spectroscopic imaging sequence using adiabatic refocusing to suppress J-modulation. In an initial MRS study of 9 patients with intractable epilepsy and 12 controls, we have observed that glutamate levels were reduced by 24% (5.1±1.2mM) in the epileptogenic region ($p<0.025$) in comparison to control (6.7±1.5mM), while NAA levels were reduced by 16% (8.0±1.1) in comparison to controls (9.5±1.0). The equivalent contralateral region showed reductions of 7% (6.2±1.6mM) and 3% (9.2±1.1mM) respectively ($p>0.05$), indicating that the reductions were specific to the epileptogenic region, as opposed to a global alteration in metabolism. Creatine levels were unchanged in the epileptogenic zone (9.7±1.1 and 9.7±1.4mM) and 6% higher (10.3±1.9mM) in the contralateral region in comparison to controls. We also have important new work showing the correlation of the decrease in NAA in neocortical epileptogenic regions with changes in electrical activity, known to be in an epileptogenic region. Eight patient and ten control MRS and MRI studies were performed in the past year.

3. fMRI: As noted previously, we have incorporated RF field map data and receiver reception sensitivity data into our routine fMRI analysis. Adjustments based on this information are important for comparison studies between MRS and/or data from subcortical electrode stimulation studies and of course for surgical planning. In the past year, we have also continued work 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 compared to the more common multi-subject analysis realm. Work is currently underway using these findings in evaluating the fMRI response in regions that demonstrate abnormal metabolic function through MRS measurements. Initial results show that
functional activity (based on fMRI) remains in regions showing NAA depletion.

4. Integrated Image Analysis: Significant progress was made in the last year related to both i.) the integration of the Yale-based image analysis software and the BrainLab image guidance platform and ii.) development of a brain shift compensation algorithm. Regarding the software integration efforts, we have developed a custom-designed client/server architecture termed VectorVision Link (VVLink) which extends functionality from the publicly-available Visualization Toolkit (VTK) . VV Link enables bi-directional data transfer such as image data sets, visualizations and tool positions in real time, permitting us to integrate research image analysis software that registers and analyzes MRS, fMRI and electrode information with the commercial BrainLab VVCranial image guidance software. The system was tested in the operating room in two surgeries so far. This was the first time our neurosurgeons could navigate in real time with such integrated multimodality data simultaneously displayed on the high resolution anatomical imaging. Work on brain shift compensation has moved toward investigating the use of sulcal features within our framework. Testing on phantoms and initial human image data is being carried out and initial results being assembled for publication.

ISSUES

All partners have been communicating effectively. The competitive renewal of this work was submitted in May.

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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, for optimizing scan rate relative to physiological motions, and for EPR imaging. 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. A second-generation scan coil power driver has been designed and constructed that produces triangular scans with scan frequencies of 1 to 20 kHz and scan widths up to 60 G. The triangle generator requires digital control commands from a computer. Bruker provided customized software to provide the digital commands (sweep frequency, sweep width, and acquisition trigger phase) to the digital triangle generator per our specifications. Triangular scans with scan rates up to 1.50×10^5 G/s have been recorded and deconvolved to yield spectra that are in good

agreement with normal slow-scan spectra, and in one prototype scan coil we achieved 20 kHz 50 G scans ($2x10^6$ G/s). These wider and faster scans have permitted us to record our first rapid-scan spectra of the complete 3-line spectra of nitroxyl radicals. These experiments provided the first direct measurement of T_2 of a nitroxyl radical at 250 MHz, 0.54 μs. Since the spin lattice relaxation time T_1 must be greater than or equal to T_2, the measurement of T_2 provides a lower limit on T_1. This is an important result because some models of the frequency dependence of T_1 predict values at 250 MHz that are much shorter than 0.5 μs.

The rapidly changing magnetic fields that are employed in the rapid scans induce eddy currents in conducting components of the resonator. These eddy currents create inhomogeneity in the magnetic field and broadening of the rapid scan signal. There is a delicate balance between the need to minimize the amount of metal in the resonator to reduce eddy currents, while still maintaining adequate shielding that reduces pickup of rf signals from the surroundings. Our latest design, which utilizes rings of multilayer PC board, is a substantial improvement over prior wire-wound or solid shields.

As the widths and scan frequencies are increased, an oscillatory background signal that is synchronous with the scan also increases. In traditional field-modulated CW EPR the baseline is flattened by phase sensitive detection. Rapid-scan EPR uses direct detection to obtain the absorption signal, so it does not have the baseline stabilization benefit of phase-sensitive detection. Since eddy currents are a major contributor to this background signal, the new design for the shield provides a substantial decrease in the oscillatory background signal. Disturbance of the resonator tuning or Q by the field sweep, such as mechanical movement of the tuning mechanism, could also contribute to the baseline signal. The baseline oscillation has been decreased by making the coupling structure as rigid as possible, and by modifying the coupling circuit.

Reconstruction of two-dimensional images by filtered back-projection (FBP) and by the maximum entropy method (MEM) was compared for the Shepp-Logan phantom and for spectral-spatial EPR images with differing signal-to-noise ratios. Each approach was found to have advantages and disadvantages. It is useful to reconstruct images using both methods and to compare the results. Two major advantages of the MEM method are that projections do not need to be equally spaced and there is not a "star" artifact. We are currently using a broadened version of the image created by FBP as a starting point for the application of MEM. A disadvantage of this approach is that the output image depends upon the extent to which the FBP image is broadened. We are examining other approaches to implementation of the MEM method.

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 has modified their data acquisition software to incorporate an option for rapid scan.

ISSUES

We are pleased with this fourth 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 (NIBIB)

ABSTRACT

We proposed a highly multidimensional approach to define and understand fundamental processes which define how the neural control and the mechanical events associated with locomotion are affected in selected motor dysfunctions . Understanding these processes is key to formulating effective intervention strategies to optimize functional motor recovery. We have developed robotic systems for quantifying and controlling the dynamics and kinematics of hindlimb stepping and standing in complete spinal rats and mice. Our experiments have demonstrated that the ability of the lumbar spinal neurons to generate stepping was dependent on the amount of robotic training in the load that was imposed during the training. The robotic device was used to control the amount of steps that ST rats performed during each training session. We also developed a new robotic training device (e.g. the "slide") that can be used to study the effects of robotic control over hindlimb coordination during training. 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. Most recently we have demonstrated that the type of control of the robotic devices plays an important role in the effectiveness of step training , in that when an "assist as needed" approach is used combined with some level of control of inter-limb coordination, the improvement in stepping is significantly better than when a fixed trajectory is imposed on the movement of the limbs. These experiments also demonstrate the effectiveness of the robots for training mice as well as rats. We have developed a Programmable Stepping Device (PSD) which can be used to train and quantify locomotor functions in spinal cord injured and stroke impaired subjects. The end products have been the development of a fully functional robotic system which can be used to train and quantify hindlimb motion in rats and mice. A prototype of a human Programmable Stepping Device is being developed which will have passed critical tests of functionality, safety, and applications for the training of locomotion in subjects with neuromuscular impairments. This prototype is

serving as the starting point for development of a commercialized product that can be used in rehabilitation clinics. These technical tasks will be developed by a team of scientists from the University of California at Los Angeles (UCLA), the University of California at Irvine (UCI) and California State University, as well as industrial participants.

STATUS OF RESEARCH AND PARTNERSHIP

We have completed most of the tasks as originally proposed. In addition we have completed several experiments that were not proposed. Objectives in the immediate future are: translate and test the current hardware and software for step training in mice to the rat, translate the newly developed device for teaching standing in the mouse to the rat, and to begin to identify some of the neurophysiological processes that contribute to the learning to step and to stand that occurs in the spinal mouse. The these developmental efforts in the studying spinal rodents has and continues to provide important insight in parallel efforts for development of devices for human subjects with spinal cord injury.

ISSUES

We have experienced little difficulty in coordinating our activities across sites. The degree of collaboration has been sufficient to fully take advantage of the unique features and contributions available from each experimental site and therefore we have been able to maintain a high level of productivity.

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PROJECT TITLE: Spectroscopic imaging and diagnosis of neoplasia

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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI)

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 built and deployed two optical probe instruments for use in the cervix and the oral cavity. These instruments are fully operational and are being used routinely. In addition, a laboratory-based clinical imaging instrument has been built and work on the clinical prototype is in progress.

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 124 subjects and the oral projects from a total of 103 subjects. These data are at various stages of analysis and correlation with pathological diagnoses, tissue morphometric parameters and HPV status.

ISSUES

None.

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PROJECT TITLE: Integrated Ultrasonic Systems for Non-invasive Therapy

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GRANTING NIH INSTITUTION/CENTER (IC): National Cancer Institute and National Heart, Lung, and Blood Institute

ABSTRACT

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

Methods. We developed an integrated ultrasonic system to position, induce, and monitor therapeutic lesions that modify various diseased tissues. Our multi-disciplinary research 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-model experiments that investigate cancer and heart-disease therapy.

Results. Our results have been incorporated in a systems model of ultrasonic therapy that facilitates comprehensive treatment planning and provides a basis for designing future therapy systems. We can control the *in vitro* size and the *in vitro* and *in vivo* location of high intensity focused ultrasound lesions with model-based predictions. We can control the size of *in vivo* lesions empirically. The size of *in vivo* lesions depends on perfusion and we are working on extracting thermodynamic parameters and extending our models to account for this.

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 PARTNERSHIPS

In previous years, we developed an integrated diagnostic/therapeutic ultrasound system. Specific Aim 5 was to test this system on animal models. This year, *in vivo* research in collaboration with Columbia and Spectrasonics was completed. In the open-chest chronic dog model, we have been able to make HIFU lesions in targeted locations including left and right ventricles and the ventricular septum. We have found that the lesions persist; after 6 weeks, the necrosed regions were replaced by fibrous tissue with no significant change in lesion size.

Specific Aim 3 was in part to establish ultrasonic monitoring techniques for sensing induced tissue changes. We continued our pursuit of this important aim, studying three promising techniques: acoustic radiation force for assessing local changes in tissue stiffness, second-harmonic imaging with B-mode clinical ultrasound machines, and spectrum analysis of second-harmonic radiofrequency ultrasound backscatter.

In previous years, we established and verified mathematical models of ultrasonically induced thermal and mechanical phenomena for modifying tissue. Specific Aim 1 was to formulate a comprehensive computer simulation embodying the mathematical models. This year, in collaboration with our partners Cornell and Spectrasonics, we have developed a modular computer model of ultrasonic techniques that characterize and modify biological tissues. We call this the Riverside Acoustic Model (RAM). Significant agreement has been found between RAM simulations, analytic solutions for simplified ultrasound propagation geometry, and experiments. RAM modules are designed to represent physical processes rather than computing routines. For example, there is a transducer module that defines the parameters of a transducer, and a lesion-formation module that delineates lesion extent based on the equivalent insonification-time model. Currently, a choice of two propagation modules is available: one for linear propagation and one for KZK nonlinear propagation based on the angular spectrum model of Dr. Christopher Vecchio of our industrial partner, Spectrasonics, Inc. In a process similar to Unix pipes, RAM modules can be connected together with a script to define a data flow. Currently, we are working to standardize data interchange among modules. Data structures will be defined with Extensible Markup Language (XML) in order to bring RAM into harmony with the newly emerging American Institute of Ultrasound in Medicine (AIUM) and International Standards Organization (ISO) therapeutic ultrasound data standards that we are advocating.

A total of seven peer-reviewed papers have been generated this year: one paper was published, three were accepted for publication, two are in the review process, and one is in preparation.

Specific Aim 6 was to formulate design procedures for future clinical systems and to identify problems requiring further investigation. We have done so with three follow-on grant applications: an R21 to explore the promising spectrum analysis of harmonics method of monitoring HIFU lesions, an R21 to explore the use of radiation force for tissue characterization with remote acoustic viscoelastography (RAVE), and a BRP in collaboration with clinical and industrial partners to develop cardiac HIFU technology for minimally invasive ventricular ablation therapies.

We have made substantial progress in meeting all six of our Specific Aims.

ISSUES

We have obtained a no-cost 1-year extension to complete studies that are underway.

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PROJECT TITLE: Ultrasound imaging and enhanced drug delivery in tumors

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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute

ABSTRACT

With the development of new biologically-active therapeutics, many of which are active at nanomolar concentrations and can be complimented by traditional chemotherapy, there is a tremendous opportunity for improvement in therapeutic efficacy. In addition, our preliminary work, as well as that of other researchers, has shown that there is great potential for ultrasound-enhanced local drug delivery. One advantage of this approach is that imaging can be coincident with therapy. We will improve delivery by exploiting ultrasound-based mechanisms that allow new therapeutics to be concentrated within a tumor, including the local release of a compound, the use of radiation force together with molecular targeting to increase the local capture of delivery vehicles, and a local increase in capillary permeability. In the first aim, we will develop wideband transducers and a 3D ultrasound system that combines imaging with a drug delivery mode, designed to enhance delivery to a specified region of interest. The system and the new tools will become available to the research community, potentially enabling research in the area of local drug delivery at many research sites, and if successful will become the basis of a new commercial system. Second, we will develop molecularly-targeted drug delivery vehicles that are localized with the use of ultrasound. With targeting ligands attached to lipid membranes on the vehicle, peptide and antibody-based targeting will be compared. Methods to increase vehicle loading will be developed. Third, we will develop and employ PET tools for the 3D quantitative assessment of the biodistribution of labeled drugs and vehicles. Fourth, we will explore mechanical, thermal and biochemical methods to increase capillary permeability and further increase local delivery. Using these sets of methods, we will conduct quantitative studies of the delivery of a chemotherapeutic to tumor models in a pre-clinical study. Although these drug delivery strategies are explored here for the unique environment of tumors, the techniques should have broad application.

STATUS OF RESEARCH AND PARTNERSHIP

We have just begun the third year of our partnerships and have made great progress toward the development of an ultrasound system, delivery vehicles, PET imaging methods, and methods to increase vascular permeability.

AIM 1- Development of transducers and an ultrasound system optimized for drug delivery: In order to simultaneously create a high resolution image and deliver a drug to a region of interest, we have

created multi-layer, multi-row transducer arrays consisting of 256 elements. Use of the multi-layer ceramic allows a much higher transmission pressure than any previous commercial ultrasound imaging array (>6 MPa). The transducer architecture is based on an inner array with a center frequency of 5.5 MHz and two outer arrays with a center frequency of 1.5 MHz, providing the opportunity to operate with these arrays from 1 to approximately 8 MHz, transmitting and receiving on individual or all arrays. These arrays have been integrated into the Siemens Antares ultrasound system with custom sequencing software, providing the opportunity to sequence between radiation force (deflection of agents), capillary permeability enhancement by mechanical effects, heating of the region to 42°C, agent fragmentation, and imaging. In the coming year, the transducer and system capabilities will be extended to three dimensions and a software interface that allows the user to specify the region of interest for treatment will be developed.

AIM 2- Development of drug delivery vehicles: Our focus in this project is to develop vehicles in which a large volume of drug or gene is incorporated in the shell of a microbubble, either by loading a large number of liposomes on the shell, using layer by layer construction to create a thick shell, or by creating a thick shell using an oil layer. In each case novel peptides or antibodies have been attached to the lipid shell for targeting the integrin $\alpha_v\beta_3$. Delivery vehicles have been loaded with the chemotherapeutic paclitaxel or with DNA/RNA for pre-clinical testing in several models. An initial therapeutic trial demonstrated a reduced tumor growth rate in the ultrasound treated tumor (compared with contralateral control).

AIM 3- Development of PET probes: We have created F18 probes to monitor the biodistribution of both the drug and the vehicle by directly labeling paclitaxel or by attaching the radiotracer to lipid molecules within the membrane. Advanced reconstruction techniques and dynamic PET methods are then used to construct high resolution images and time activity curves. Initial use of these probes have provided the opportunity to optimize the lipid content, agent size, and targeting ligands.

AIM 4- Increase capillary permeability: Thermal and mechanical methods to increase capillary permeability have been developed and are being evaluated in preclinical trials. Local delivery can be enhanced up to three fold compared with surrounding regions using these methods. The mechanisms for the increased permeability are under evaluation including assessment of heat shock expression and neutrophil recruitment.

ISSUES

The partnership has been very successful with a three-way intellectual property in place and terrific cooperation between the partners. Several of the methods show promise to independently increase local delivery by 3 to 15 fold and preclinical testing is now underway to combine the various ultrasound methods with molecular targeting. The development of optical and PET imaging surrogates has proven to be invaluable in the evaluation of the various mechanisms for enhanced delivery.

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PROJECT TITLE: Intraoperative Near-Infrared Fluorescence Imaging

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NIH GRANTING INSTITUTE/CENTER: National Cancer Institute

ABSTRACT

The major hypothesis guiding this study is that near-infrared (NIR) fluorescence imaging has the potential to improve human cancer surgery by providing sensitive, specific, and real-time intraoperative visualization of normal and disease processes. The PI's laboratory has developed a low-cost, safe, and easy to use NIR fluorescence imaging system that permits the surgeon to see surgical anatomy and invisible NIR fluorescence simultaneously, in real-time, and with high spatial resolution. To translate this technology to the clinic, we have established a Bioengineering Research Partnership (BRP) comprised of members of the PI's laboratory, including human surgeons, and our industrial partner GE Global Research. GE is committed to translating the imaging system to the clinic, and will provide their extensive expertise in biomedical system design and implementation. GE and the BIDMC have also signed a model agreement concerning intellectual property and shared development, which ensures that academic and industrial resources are fully leveraged during technology translation.

The end-point of our study will be the development of an intraoperative NIR fluorescence imaging system ready for human clinical trials. To achieve this goal, we describe a systematic series of small and large animal studies, and a closed-loop feedback development process designed to optimize each system component. The timeline for our study is as follows: Project Year (PY) 1 - initial design of the prototype imaging system for open surgeries. PY 2 - addition of endoscopy/laparoscopy capabilities for minimally invasive surgery. PY 3 - addition of optical diffusion technology to the open surgery system. PY 4 - final prototype development for the open and minimally-invasive imaging systems, and PY 5 - final optimization of system components and software, final validation studies, and preparation for translation to the clinic.

Immediate cancer surgery applications of the imaging system include image-guided sentinel lymph node mapping, image-guided cancer resection with real-time assessment of surgical margins, and intraoperative detection of occult metastases in the surgical field. The imaging system will also ensure that critical structures such as nerves and blood vessels are visualized and avoided. Taken together, this BRP application describes an academic/industrial partnership engineered for successful translation of a general-purpose optical imaging technology to the clinic.

STATUS OF RESEARCH AND PARTNERSHIP

During the first year of this grant, we have successfully completed Specific Aim 1, the design and construction of a complete intraoperative near-infrared (NIR) fluorescence imaging system for open surgery. Two major engineering challenges were overcome in realizing this system: 1) simultaneous dual-channel excitation/emission NIR fluorescence imaging with no moving parts, 2) a low-profile, high-powered non-laser, LED-based light source for surgical imaging.

Our newest design for the system optics utilizes two dichroic mirrors in series, which direct color (400-670 nm), NIR emission #1 (683-717 nm), and NIR emission #2 (>800 nm) light to three different cameras. This flexible design permits simultaneous excitation and simultaneous emission monitoring of two different NIR fluorophores as well as color video (i.e., surgical anatomy).

In order to make surgical imaging a reality, we needed to develop a low-profile, high-power, non-laser based light source. The light source must also be fully contained within a sterile drape and be capable of achieving fluence rates of up to 25 mW/cm2 for exciting two different fluorophores, as well as white light for illuminating the surgical field. As detailed on our poster, we achieved these design specifications by employing over 1,000 LEDs in a low-profile unit comprised of 54 1"x1" modules, each housing 20 LEDs, driven by custom-designed, scalable electronics. Given the extremely high heat load generated by these LEDs, a Fluorinert FC-3283 cooling system was designed, which permits close-circuit, remote monitoring and control of light source temperature. The entire unit is mounted in an aluminum housing 11" wide by 9.75" deep, and only 4.75" high, and is contained within a sterilizable NIR-transmissive shield and drape.

Using these engineering advances, we continue to develop novel techniques for real-time surgical guidance. Our poster will highlight recent advances in the detection of intravascular thrombi using two-color NIR fluorescence, and the use of simple tetrasulphonated fluorophores for identification of ureters and common bile duct during common human surgeries. In addition, the chemists on our team continue to improve targeted NIR fluorophores for image-guided cancer resection.

ISSUES

None. Our model of academic/industrial collaboration, based on a prospective intellectual property and shared development agreement signed by both parties, appears to be working extremely well.

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

PARTNERS' NAMES AND AFFILIATIONS:

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Minnetronix, Inc. (St. Paul, Minnesota)

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

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

RVAD Mechanical System Progress: *In vitro* performance testing of the DexAide RVAD has met design criteria, and the nominal operating condition of 4 L/min and 20 mm Hg pressure rise was achieved at 2,000 rpm with a power consumption of 1.9 watts. The calf inflow cannula design is still evolving to minimize depositions on the cannula observed in most experiments. This past year's effort in developing the RVAD's mechanical system has been directed towards (1) Refinement of the beta prototype, (2) Fabrication of four production-like volute housings, (3) Fabrication of four welded and seamless rotating assemblies, representing a production-like manufacturing process, (4) Fabrication and *in vivo* evaluation of three more new inlet cannula configurations, and (5) Design data for the RVAD pump and outlet fitting are available for technology transfer.

RVAD External Electronics Development: The design and development of external electronics have been completed for the stand-alone RVAD system, and verification tests are under way in preparation for preclinical tests. Work on the external electronics design for the biventricular assist system is ongoing.

RVAD Computational Fluid Dynamics (CFD) Study: The computational fluid dynamics (CFD) effort focused on establishing a force-balanced position of the rotating assembly. Numerous CFD analyses were performed of the thin, blood-lubricated journal bearing section to determine the radial position of the rotating assembly where the magnetic bearing forces were directly offset by the hydrodynamic fluid generated forces.

RVAD In Vivo Testing and Fitting Study: Short-term experiments intended for a 2-week duration (n = 5) or 1-month duration (n = 6) have been performed to date. Hemodynamics were stable in all calves with a mean measured pump flow of 4.9 ± 0.8 L/min. The pump speed and power consumption were 2,533 ± 104 rpm and 3.3 ± 0.3 watts, respectively. All 11 calves were terminated electively; however, 5 calves were sacrificed not at the completion of the planned duration, but prematurely due to inflow cannula obstruction and pump depositions. Fitting studies were performed in 5 cadavers and 3 patients who underwent an LVAD implantation. Implantation of the DexAide in the pre-peritoneal space was possible with a model of the CorAide LVAD but interfered with a model of the HeartMate LVAD or Novacor LVAD. The DexAide fit well within the right chest cavity when implanted with a CorAide, HeartMate, or Novacor LVAD.

Clinical Data Analysis of BVAD Patients: Recognition of risk factors for mortality after BVAD implantation is important for development of the devices, patient selection, and optimal outcomes. The hemodynamic data from 44 clinical patients who underwent BVAD implantation were analyzed. The data revealed that BVAD implantation still remains one of the challenges in treatment of severe heart failure. Prior cardiac surgery and elevated creatinine were risk factors for mortality after BVAD implantation.

ISSUES

Due to the delay in the delivery of the pre-clinical RVADs and also due to the inflow cannula problems observed in the 1-month RVAD experiments, there will be a delay in pre-clinical testing.

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PROJECT TITLE: Molecular Analysis of Visual Processing

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GRANTING NIH INSTITUTE/CENTER: National Eye Institute (NEI)

ABSTRACT

We are developing 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 tools to measure and to alter gene expression in the monkey visual cortex to study the role of signal transduction networks 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, and reversible inactivation of specific cell classes using molecular tools. Once developed, these techniques will facilitate our 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 the role of specific neural circuits in perception and behavior.

STATUS OF RESEARCH AND PARTNERSHIP

Monitoring and manipulating gene function in monkey visual cortex. We are combining laser capture microdissection (LCM) with hybridization to our own spotted microarray based on a cDNA library prepared from monkey visual cortex and to the newly develop Affymetrix macaque array. We have also perfected in situ hybridization and RT-PCR to confirm and follow up the microarray data. Determining the role of specific genes in cortical function also requires perturbing the expression of these genes. To this end we are applying RNA interference (RNAi) as a means towards manipulating gene function in monkey visual cortex. This included developing viral vectors for delivering constructs to induce interference.

Genetic methods for inactivating visual pathways. Genetic strategies for perturbing activity of selected neurons hold great promise for understanding circuitry and behavior. Several such strategies exist, but there has been no direct demonstration of reversible inactivation of mammalian neurons in vivo. We previously reported quickly reversible inactivation of neurons

in vitro using expression of the Drosophila allatostatin receptor (AlstR). In our current work, adeno-associated viral vectors are used to express AlstR in vivo in cortical and thalamic neurons of rats, ferrets, and monkeys. Application of the receptor's ligand, allatostatin (AL), leads to a dramatic reduction in neural activity, including responses of visual neurons to optimized visual stimuli. Additionally, AL eliminates activity in spinal cords of transgenic mice conditionally expressing AlstR. This reduction occurs selectively in AlstR expressing neurons. Inactivation can be reversed within minutes upon washout of the ligand, and is repeatable, demonstrating that the AlstR/AL system is effective for selective, quick and reversible silencing of mammalian neurons in vivo.

Viral vectors for gene delivery. We have successfully used AAV for obtaining long-term expression of genes in the monkey cortex. We are also developing other viral vectors, including lentivirus and rabies virus for the study of cortical circuits.

ISSUES

None.

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PROJECT TITLE: Development/Testing of Artificial Retinas for the Blind

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Dr. E.J. Chichilnisky, Salk Institute

GRANTING NIH INSTITUTE/CENTER: National Eye Institute (NEI)

ABSTRACT

Our research objective for this partnership grant has been to develop a long-term implantable retinal stimulator for patients blinded by outer retinal degenerations. Using technologies developed by the Alfred E. Mann group of companies over the past 30 years for implantable stimulators, we are developing a chronic retinal stimulator and associated external hardware to be used for use both basic research and as a clinical device.

To achieve this goal, several areas of research are still needed. In this bioengineering research partnership, academia collaborates with industry to accomplish the basic research necessary to make a chronic retinal prosthesis a reality. Areas of research that we focus on include:

- Electrode geometry and electrode material selection
- Surgical attachment of the retinal implant
- Low power electronic circuit design
- Hermetic implantable packaging

Each of these areas needs additional research for the creation of an optimal chronic retinal prosthesis that will enable persons blinded by outer retinal degenerations to regain the most important loss they have suffered—the loss of mobility. The aim of this five-year proposal is to complete the design and manufacture of a retinal prosthesis and associated external hardware and test it chronically in animals, so that an investigational device application can be made to the FDA in preparation for a clinical trial.

STATUS OF RESEARCH AND PARTNERSHIP

The partnership continues to lead the field of retinal prosthetics, well ahead of our original schedule. First generation implants with 16 independently controllable electrodes which were developed under this program continue to perform well in patients for over four years now, with the patients using the device daily at home. Technology development for a second generation implant with 60 independently controllable electrodes is complete and prototype units of second

generation implants have been built. Extensive bench and animal data, which is nearing completion, will be used soon to support clinical studies with second generation devices. A third generation device is under development which is expected to yield a clinically useful prosthesis.

ISSUES

The current group continues to work well together in a highly productive manner. There are no open issues.

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PROJECT TITLE: Self Assembling Peptides for Tissue Engineering

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

ABSTRACT

A fundamental challenge in tissue engineering is the nature and design of an appropriate 3D scaffold. We propose to use self-assembling peptides to engineer the 3D environment of cells with biologic functionality that can be modified and controlled, based on the basic biophysics of the material, which can be tailored for specific cell types. We employ a Partnership that brings together investigators in biophysics, bioengineering, cell biology, molecular biology, physiology, chemistry, and imaging. Team members are specialists in Electrical Engineering (Grodzinsky, PI), Mechanical Engineering (Kamm), Chemical Engineering (Griffith), Biological Sciences (Zhang, Semino, Lee-BWH), Chemistry (Klibanov) and Clinical Science (Lee, Frisbie-CSU). The use of self-assembling peptides in tissue engineering potentially enables the control of cellular adhesion, biomechanical properties, growth factor presentation and/or release, and vascularization. A fundamental theme of this Partnership is that no single tissue engineering approach is suitable for the diverse structure of all tissues. However, our central hypothesis is that by providing a physiologically appropriate, molecularly specific environment that can be modified by design, we can utilize the "core technologies" of the Partnership to improve the approach for a given tissue. This Partnership brings together expertise in several specific tissues, allowing us to interact in ways that traditional individual grants and programs do not provide. Our Specific Aims are (1) Design, & functionalization of peptide sequence of self-assembling peptides for 3D tissue engineering; (2) To explore the basic biophysics of the self-assembling peptide environment using state-of-the-art computational modeling and biophysical measurements; and (3) To explore the role of the self-assembling peptide environment in three major target tissues: myocardium, cartilage, and liver. The lead institution of the Partnership is MIT, with partners from BWH and CSU.

STATUS OF RESEARCH AND PARTNERSHIP

We are now in the 10th month of the first year of our grant. The Partnership is working extremely well together. We have monthly meetings attended by all the faculty members and, typically, about 3-4 students and/or Post-Docs from each of the partnership-faculty groups. These workshops consist of either presentations by core faculty members, or more detailed workshops on a particular theme that is requested by the team. All but one of the groups are based in Boston. The PI has met with the group members from Colorado State University at two conferences this past year; in addition, the PI visited the CSU team at their home base in Fort Collins for a 3-day meeting specifically devoted to ongoing collaborative studies between the CSU team and the Boston area partners.
Research is ongoing in all aspects of the project. (Details have been submitted in the form of the Year-1 Progress Report to NIH.) Several publications coming from the partnership collaborations are now either in the published or submission stage.

ISSUES

One issue that has been under discussion amongst the Partnership investigators concerns the staging of certain animal studies. In particular, the rabbit and equine studies focusing on cartilage tissue engineering had originally been scheduled to begin in Year 3 of the Grant. However, due to excellent progress on many fronts, team members at CSU, MIT, and BWH would like to initiate these studies in Year 2. A final determination and recommendation will be forthcoming, based on the results of in vitro studies currently underway.

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PROJECT TITLE: Magnetic Resonance Guided Electrophysiology Intervention

PARTNERS:

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2) Robin Medical, Incorportated (Balimore, MD)
3) Irvine Biomedical Incorporated (Irvine, CA)
4) Navicath, Incorporated (Haifa, Israel)

GRANTING INSTITUTE: National Heart, Lung and Blood Institute (NHLBI)

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, and successfully performing studies in 2 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 (6) Approval of the competing renewal of the project for another 5 years.

Our Investigational Device Exemption (IDE) 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. We have performed studies in 2 patients under our IDE, in patients after atrial flutter ablation. Diagnostic quality electrogram signals were obtained, and there were no adverse effects noted, indicating that real-time MRI guided electrophysiology studies are feasible.

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 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.

Magnetic resonance imaging (MRI) is an important diagnostic modality currently unavailable for millions of patients due to the presence of implantable cardiac devices. We evaluated the diagnostic utility and safety of non-cardiac and cardiac MRI at 1.5 Tesla, when using a protocol that incorporates device selection and programming, and limits the estimated specific absorption rate of MRI sequences. This evaluation is important in the context of this project as this patient population is more likely to require electrophysiology intervention. We performed 102 scans in 85 patients and measured device parameters before the scan, immediately after the scan, and then again at 3 months after the scan. We found no significant differences in atrial or ventricular electrogram amplitudes, lead impedances, or pacing capture thresholds, indicating that MRI scanning can be performed safely in patients with selected devices.

We developed a prototype of an MRI compatible, steerable EP catheter that includes tip tracking electronics. The catheter is constructed of PEBAX and is EMI shielded throughout most of its length to allow better EP signals and reduce RF reception. An open loop design was used for tip tracking and shaft visibility. Electronic circuits to separate the MRI from the EP signals is housed in the handle.

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: Tissue Engineering of Hematopoietic Bone

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Thomas Zwaka, MD/PhD (Center for Cell & Gene Therapy, Baylor College of Medicine)
Michael Barry , PhD (Center for Cell & Gene Therapy, Baylor College of Medicine and Dept. Bioengineering, Rice University)

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

ABSTRACT

Our long-term goal is to engineer hematopoietic bone ex vivo to treat disorders of both bone and hematopoiesis. The specific goal of this application is to produce bone that contains marrow with pluripotent, repopulating stem cells that can fulfill the long-term regenerative needs of patients, as well as provide structural integrity for the repair of bone defects. To achieve this goal, we have assembled a team of interactive investigators from Baylor College of Medicine (BCM) and Rice that have the required expertise in biology and engineering, which includes: hematopoiesis and stem cell biology (Goodell and Zwaka, BCM), bone development (Davis, BCM), vascular development (Hirschi, BCM & Rice), biomaterials and bioreactors (West and Mikos, Rice) and bioimaging (Sevick-Muraca, Dickinson and Barry, BCM and Rice). Our overarching hypotheses are that the steps that lead to bone formation and the establishment of functional marrow and vasculature are dissectible and definable in a model of de novo bone formation; furthermore, by understanding the sequence and kinetics of the cellular and molecular events needed for this process, we will gain insight into how to recapitulate hematopoietic bone formation ex vivo for the propagation of pluripotent HSC in vitro and in vivo. Toward addressing these hypotheses, we have established a model of de novo bone formation in which vascularized, marrow-filled bone was generated in vivo, and demonstrated that the marrow formed within this bone structure enables the survival and propagation of functional HSC that are capable of long-term reconstitution of all blood cell lineages in vivo. We have begun the dissect and define the molecular steps that lead to hematopoietic bone formation and have established bioimaging techniques needed to track the fate and function of marrow-derived cells ex vivo and in vivo. We have designed and generated biomaterials that will enable cellular survival and propagation, and bioreactors in which bone and blood vessels are readily fabricated. In our ongoing BRP project, we are working towards integrating all of these components to engineer

hematopoietic bone and test its functions in vitro and in vivo. Furthermore, we have established necessary links to BCM and Rice technology transfer offices to facilitate the transition of our research into biotech and clinical settings.

STATUS OF RESEARCH AND PARTNERSHIP

Current State of Our Research Project: We have made significant progress toward addressing the first three of four Specific Aims that we proposed.

Effectiveness of Our Partnership: To facilitate sharing of data and ideas among the many research groups involved in this project, and needed for its success, we have established formal monthly meetings in which all investigators and trainees participate. At each meeting, two lab groups present their data, discuss problems/issues that they are dealing with, and get valuable and necessary feedback from all of the other groups. This meeting format has been particularly useful for identifying and establishing collaborative efforts needed to tackle complex, interdisciplinary problems as they arise during the evolution of our project. For example, using this strategy, our group has realized significant cellular sensitivity problems with our proposed bio-imaging techniques (as described above). When we tried to track small cell numbers in vivo, as needed for tracking of hematopoietic stem cells, we found that there was too much background autofluorescence impeding the visualization of the cells. To address this issue, our group discussed alternative strategies, and incorporated new techniques that should prove to be most sensitive and effective for tracking small cell numbers. The bio-imagers are working closely with the stem cell biologist to optimize the new strategies.

In summary, the individual groups involved in this project are generating needed data in their respective areas of expertise, and all research groups are working effectively as a team to efficiently address multidisciplinary issues as they arise.

ISSUES

Our team has been required to change personnel. Dr. Michael Barry will be leaving Baylor College of Medicine, and it will be impossible for him to continue his proposed imaging experiments because he will not have access to the specialized, custom-built imaging system needed. Moreover, it has become increasingly evident that the dynamic bioluminescence imaging system under development by Dr Barry was insufficiently sensitive to track the small numbers of HSC present in vivo.

To circumvent this problem, Dr. Barry had also begun working with Dr. Eva Sevick-Muraca to expedite the in vivo tracking method initially proposed as sub-Aim 2D of our BRP grant proposal. In this alternate approach, the injected cells are labeled with a halo-tag, which is a near infrared dye and can be imaged at wavelengths that have far less autofluorescence. This lower background produces greatly increased sensitivity, enabling tracking of far fewer cells in vivo. The spectral range of the far infrared dye is also amenable to microscopy. To take advantage of this property, and to enable dynamic imaging via confocal and multi-photon microscopy, we will be adding Dr. Mary Dickinson to our research team to replace Dr. Barry. She is a new faculty member at Baylor, and expert in this type of dynamic bio-imaging.

We will also add Dr. Thomas Zwaka to our team, who is an expert in the cultivation and genetic manipulation of human embryonic stem cells. He was not yet a faculty member at Baylor when this application was submitted. However, we believe that we need to include his expertise in generating needed human cell types for future clinical studies involving tissue engineered hematopoietic bone for human patients.

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PROJECT TITLE: Quantitation of Cellular Protein Production in Real Time

PARTNER'S NAMES AND AFFILIATIONS:

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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 cell phenotype including 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 hematopoietic stem cells and accessory cells including 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 the hematopoietic stem cell niche using human bone marrow stromal cells and osteoblasts in an in vitro model system.

STATUS OF RESEARCH AND PARTNERSHIP

In the fourth year of this partnership we have more fully incorporated using hematopoietic stem cells in co-culture with stromal cells as an archetype model for the stem cell niche. This model takes advantage of expertise with stem cells from UPCI and anticipates incorporation of osteoblasts into the model to build on strengths of the HMS collaboration. We are nearing completion of a first-of-its-kind study of purified stem cells during the early stages of cobblestone area formation, as a well-characterized starting point for the hematopoietic stem cell niche (Song, YF et al, Stem cell traits as revealed by time-lapse imaging in long-term co-culture, and Bahnson, AB et al, Migratory behavior of hematopoietic stem cells leading to cobblestone area formation, in preparation). The analysis was performed using a variety of custom modifications for better determination of family lineage of progeny from rare sorted individual

cells tracked during their migration and interactions with stromal cells. Inter-division time analysis was used to explore traits expected in successful niches such as quiescence and asymmetry between sisters indicating self-renewal plus repopulating expansion. New tools and concepts for analysis have been developed as a result of this study, including interactive software for tracking and lineage analysis and for assembly of adjacent view-field mosaics for high resolution tracking across long migration paths within a unified global coordinate system. Bone marrow from GFP-transgenic mice was used for donor cell discrimination, requiring new correction and normalization algorithms and multi-folder processing routines for high levels of auto-fluorescence from primary stromal cells.

Major architectural modifications were made to the system operation software in order to better accommodate plug-in type accessory software and devices, such as multi-unit fluidics needles and pump drives for our cell surface marker staining objectives. Similar changes are being made to the image analysis software that will ultimately facilitate the goals for dynamic manipulation of cells based upon real time analysis through complete integration of these components. Improvements have also been made to the operating software to enable on-the-fly input of coordinate modifications that give the user expanded options for designing/changing plate layout patterns and for performing 3D focal position transformations with commercial spreadsheet software in addition to using the custom auto-focus algorithms. We have built a prototype fluidics needle drive that incorporates a new concept for remotely locating the motors in multi-needle arrays in order to be able to service multi-well plates (up to 384-well type) row-by-row, with the option to apply independent parameters to each individual well. For off-line analysis of secreted proteins captured on beads, we have demonstrated selective recovery of pre-identified beads of interest in 384 well plates, and have successfully transferred them to external containers for analysis by mass spec. A new design for the plate lid remove/replace device is nearing completion.

In addition to the hematopoietic stem cell niche investigations, the team at Hillman Cancer Center has performed automated cell tracking and quantitation of cell motility leading to additional publications focused on the Smad3 gene, using knockouts and retroviral vector-mediated gene replacement. The Smad family of proteins are crucial components of the TGF-beta signaling pathway and smad3 has an important role in bone formation by osteoblasts. Smad3 is also an important effector of the TGF beta mediated inhibition of osteoblast differentiation, enhancement of alkaline phosphatase activity, mineralization, and bone matrix proteins such as collagen I and osteopontin. We studied clonal bone marrow stromal cell lines from Smad3-/- and Smad3+/+ littermates for migratory capacity. We also utilized several parameters of measurement of radiation sensitivity of these clonal bone marrow stromal cell lines, and a subclonal line of Smad3-/- cells into which the Smad3 gene was reinserted. Smad3-/- mice display decreased ionizing irradiation-induced skin fibrosis suggesting a defect in fibroblast proliferation and migration. The results show reduced in vitro and in vivo migratory capacity of Smad3-/- bone marrow stromal cells. Reinsertion and expression of the Smad3 gene restored each biologic property to levels close to those observed with Smad3+/+ cells. The decreased migratory capacity of Smad3-/- cells in vitro correlate with decreased radiation fibrosis in vivo in mice deficient in TGF-beta signaling. The data show that the reduced in vitro and in vivo migratory capacity of Smad3-/- bone marrow stromal cells correlates with decreased radiation pulmonary fibrosis observed in mice chimeric for Smad3-/- marrow and help explain the decreased radiation fibrosis and reduced acute injury response reported in irradiated Smad3-/- mice

ISSUES

As indicated in our last year's report, we are pursuing a variety of approaches for extracting useful data, including manual methods when necessary for measuring motility and growth characteristics of cells in situations of low-contrast and for co-culture analysis where the tracking rare cells is beyond the current capabilities for automated image analysis methods.

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PROJECT TITLE: Advanced Imaging for Glaucoma

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GRANTING NIH INSTITUTE/CENTER: National Eye Institute (NEI)

ABSTRACT

Glaucoma is a leading cause of blindness that presents a considerable diagnostic challenge. It is a chronic degeneration of the retinal nerve fibers most often associated with elevated intraocular pressure. However, the level of intraocular pressure by itself is a poor predictor of eventual visual field loss. Visual field testing is a late indicator of glaucoma and suffers from poor sensitivity and reproducibility. Studies have shown that, in glaucoma, up to half of the retinal nerve fibers can be lost before visual field loss is detected. Our goal is to improve glaucoma diagnosis with optical coherence tomography (OCT) and other new imaging methods that can reveal tissue and cell-level structures in the retinal layers affected by glaucoma.

OCT is a novel technology that provides cross-sectional retinal images with micron level resolution, which is not possible with any other non-invasive method. It has been used to measure the peripapillary retinal nerve fiber layer (NFL) thickness. Although NFL thickness correlates well with conventional diagnostic indicators, there is still considerable overlap between glaucomatous and normal eyes. We propose to go beyond NFL thickness measurements and develop OCT-based technology to measure internal NFL properties such as reflectivity, birefringence, and backscattering angular distribution. We will also develop high-speed and ultrahigh resolution OCT to allow direct measurement of the much thinner ganglion cell layer (GCL).

University of Southern California (USC), the lead institution, will work with partners at Duke Univ. and Case Western Reserve Univ. (CWRU) to develop the advanced OCT instruments. Initial instrument validation will use animal models of glaucoma developed at the University of Miami Bascom Palmer Eye Institute (BPEI). The new instruments will be tested in a 5-year clinical trial at USC, Cleveland Clinic Foundation (CCF), Univ. of Pittsburgh Medical Center (UPMC) and BPEI. High speed OCT will be used in year 1 with other OCT technologies to be introduced to the clinical trial in years 2-4. OCT will be compared with other existing advanced imaging technologies such as scanning laser tomography and polarimetry. The greatest portion of the trial will assess the ability of advanced imaging to predict which ocular hypertensive

patients will later develop glaucoma as defined by conventional visual field and optic disc evaluation. If these advanced imaging technologies can predict glaucoma development, then they may allow earlier treatment and prevention of visual loss.

STATUS OF RESEARCH AND PARTNERSHIP

The project is near the end of Year 3 of 5. The Advanced Imaging for Glaucoma Study (AIGS) has enrolled 224 eyes in the Normal group, 260 eyes in the Glaucoma Suspect and PrePerimetric Glaucoma group and 196 eyes in the Perimetric Glaucoma Group.

The AIG team has developed and/or tested the following novel imaging software and hardware designed to improve glaucoma diagnosis:

1. The USC team developed software to measure the inner retinal layer (IRL = nerve fiber layer+ganglion cell layer+inner plexiform layer) in the macula using the high-speed Stratus OCT (Carl Zeiss Meditec, Inc., Dublin, CA). The diagnostic power of macular inner retinal layer thickness was found to be superior to total retinal thickness and equivalent to circumpapillary nerve fiber layer (cpNFL) thickness.
2. The USC team developed a new macular grid scanning pattern for the Stratus OCT. The diagnostic power of IRL thickness was further improved and found to compliment cpNFL thickness in detecting abnormality in the GSPPG group.
3. Zeiss provided enhance corneal compensation (ECC) software to improve the signal-to-noise ratio of NFL birefringence measurement with scanning laser polarimetry (GDx-VCC). ECC was found to diagnose glaucoma more accurately in eye with atypical birefringence pattern due to retinal pigment epithelial atrophy and increased backscattering from deeper layers.
4. The Duke team developed an ultrahigh speed (26,000 axial scans per second) high-resolution (6 microns) Fourier domain (FD) OCT system. FD-OCT is now routinely used in the AIGS at the USC and UPMC clinical centers for optic nerve head, cpNFL, and macular scanning. Preliminary results indicate improved repeatability and diagnostic power compared to Stratus OCT.

The CWRU team has developed an OCT system that scans incidence angle. Results from *ex vivo* retina measurements were able to characterize NFL directional reflectance. But due to technical difficulties we will not pursue a clinical instrument based on this technology. A dual-angle Doppler OCT technology was also developed and 3 dimensional flow vector measurement was demonstrated. We will further develop this for retinal blood flow measurement.

The Duke team is developing a polarization sensitive FD-OCT system for measuring NFL birefringence changes in glaucoma in a rat model developed by the BPEI team. An equivalent clinical system is being developed at USC.

ISSUES

The recruitment of patients was slower than anticipated and completion of goals will require the full 5 years rather than the initial estimate of 2 years. Some longitudinal study goals will require renewal and extension of the clinical study to be accomplished. The development of ultrahigh speed FD-OCT technology was on schedule but the development of polarization-sensitive OCT was delayed and multi-angle OCT was abandoned after initial testing. Dual-angle Doppler OCT was adapted as a new goal due to encouraging data.

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

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

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 project, we have focused on molecular and cellular mechanisms of vascular adaptation in coronary and cerebral arteries and arterioles, and the associated integrated manifestations in vessel morphology and function at cellular and tissue levels. Toward this end, we have developed a new micro-pig model of systemic hypertension that allows us to detail the time-course of hemodynamic changes during the development and reversal of hypertension. Using an externally controllable suprarenal aortic coarctation model, we have focused on mechanical effects while exploring 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, however, 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 hypothesized that altered stresses (intramural circumferential and axial as well as 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 myogenic responses

collectively result in bed-specific adaptations. These hypotheses are being tested by combining clinical, molecular, cell biological, immunohistochemical, morphological, and biomechanical methods to study coronary and cerebral vessels (n = 5-8 per cohort) at multiple times (2, 4, 6, and 8 weeks) during the development and reversal (8 weeks) 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 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.

STATUS OF RESEARCH AND PARTNERSHIP

We continue to make significant progress along two primary lines: further development of new tools (computational and algorithmic) for data analysis and collection of new data on cellular, microstructural, and biomechanical changes in multiple vascular beds due to hypertension. With regard to the former, we have 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 and can incorporate directly information on the kinetics of cell and matrix turnover. In particular, by coupling mixture theory equations for soluble and insoluble (structurally significant) constituents, we have achieved our goal of a biomechanical framework that can incorporate experimentally accessible data on much of the biology. Although not originally proposed, we have shown that this theoretical framework can also capture some aspects of cell adaptation to altered mechanical loading. We feel this could be a major advance, particularly as the biomechanics community moves towards multi-scale modeling. We also implemented these models in custom finite element codes, which will provide even greater applicability. We also continue to make significant progress in developing 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). It is becoming increasingly clear that, as proposed, arterial and arteriolar responses depend on both the axial and the circumferential stress. We completed the first biaxial study of isolated arterioles. Although basic function is nearly insensitive to ±10% changes in axial stretch, this stretch dramatically affects the biaxial stresses and thus signals for growth and remodeling. Finally, note that our data on the effects of pharmacologic reversal of the hypertension will not be completed (each takes ~20 weeks in the micro-pig) by the scheduled end of the first 5-year study period; hence, we have no-cost extended the project to enable sufficient time for full data analysis.

ISSUES

One partner moved this summer from Texas A&M University to another university and decided to leave the partnership. His Ph.D. student has graduated and remained as a Veterinary Surgical Resident, however, and will complete some of the planned data analysis. This situation was unexpected, but will not impact significantly the overall goal. Because we added one new partner last year, the total number of originally proposed partners remains.

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PROJECT TITLE: Integrative Biology of Tumor Metastasis

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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI)

ABSTRACT

The unprecedented advances made in current understanding of the molecular origins of cancer have not translated into a commensurate decrease in mortality from metastatic disease. In our judgment, a more complete understanding of the integrative biology of metastasis is essential for the development of novel and effective approaches to cancer treatment. In this Bioengineering Research Partnership program, we use a systems approach to uncover molecular, cellular and physical mechanisms governing metastasis, and we use mathematical modeling to integrate the results. To this end, we have built a multidisciplinary team of Harvard-MGH bioengineers, tumor biologists and clinicians (as well as 30 collaborative partners) with a successful track record of integrative basic and translational investigations. Results from this team over the past 3.5 years led to novel bioengineering innovations (Nature Medicine, 2001, 2003, 2004-a), a comprehensive mathematical model of cell bio-distribution (Blood, 2003) and

exciting scientific findings (PNAS, 2001; Science, 2002; Nature, 2004-a) that suggest compelling new hypotheses regarding tumor-host interactions and metastases.

Specifically, in Project 1 (D. Fukumura, MD, PhD), we revisit the century old "seed and soil hypothesis" and suggest a new paradigm for the role of stromal cells in metastasis. In Project 2 (Y. Boucher, PhD), we aim to explain the important clinical observation that defects in collagen synthesis correlate with increased metastasis, and to identify new therapeutic targets in the collagen matrix. In Project 3 (L.L. Munn, PhD), we investigate effects of mechanical stress on tumor progression and metastasis – an important yet unexplored area of research. Finally, in Project 4 (R.K. Jain, PhD), we utilize a clinically relevant model of distant disease to control both lymphatic and blood-borne metastases by blocking VEGFR1, –R2 and –R3 pathways.

STATUS OF RESEARCH AND PARTNERSHIP

We have made significant progress in all Projects or our BRP. For example, in the lymphatic project, we have developed novel approaches to imaging and tracking lymphatic metastasis. Such models are necessary to study how molecules such as vascular endothelial growth factor (VEGF)-C affect the incidence of lymph node metastases. In these studies, we imaged and quantified lymphatic metastasis in tumors growing in the tip of the mouse ear using intravital microscopy of the draining lymphatic vessels and lymph node, which receives spontaneously shed tumor cells. We found that VEGF-C overexpression in cancer cells induces hyperplasia in peritumor lymphatic vessels and increases the volumetric flow rate in lymphatics at the base of the ear by 40%. The increases in lymph flow rate and peritumor lymphatic surface area enhance the rate of tumor cell delivery to lymph nodes, leading to a 200-fold increase in cancer cell accumulation in the lymph node and a 4-fold increase in lymph node metastasis. In our model, VEGF-C overexpression does not confer any survival or growth advantage on cancer cells. We also found that an anti-VEGF receptor (VEGFR)-3 antibody reduces both lymphatic hyperplasia and the delivery of tumor cells to the draining lymph node, leading to a reduction in lymph node metastasis. However, this treatment is unable to prevent the growth of tumor cells already seeded in lymph nodes. Our results indicate that VEGF-C facilitates lymphatic metastasis by increasing the delivery of cancer cells to lymph nodes and therapies directed against VEGF-C/VEGFR-3 signaling target the initial steps of lymphatic metastasis. This study exemplifies how our partnership is developing novel technologies and applying them to timely questions in the field of metastasis in order to enable more effective therapies.

ISSUES

There have been no significant issues with the technical, administrative, or programmatic aspects of the partnership.

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

PARTNERS' NAMES AND AFFILIATIONS:
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GRANTING NIH INSTITUTION/CENTER: National Institute of Child Health and Human Development (NICHD)

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. This abstract summarizes the work conducted over the past five years. The specific aims for the project involved *in vivo* screening of various design parameters independently in a rat model. The final prototype engineered from the optimal design is being 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, biomechanical stability, 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. A one centimeter defect of rat sciatic nerve was used as the animal model. The *in vivo* screening studies have been completed and the data analyzed. The final prototype, based on the optimal design parameters, is currently being evaluated in a primate model at Duke University. The 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 (NG_{hp}) has a MW cut-off at $2x10^6$ Daltons and low permeability prototype (NG_{lp}) has a MW cut-off at 16,000 Daltons; Nerve guides with 2 types of guiding channels, micro-tube (NG_{mt}) and filament (NG_{mf}) guiding channels; NG_{mt} 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 NG_{hp} group over NG_{lp} group. After 24 weeks, the NG_{hp} showed a strong positive correlation between the number of MA and the luminal nerve guide area. In the NG group with guiding channels, axonal regeneration is very robust in NG_{mt} group. 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 NG_{mt} supported the histomorphometrical studies (50% of the AG

group at 6 weeks). However, in the NG_{mf} 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.

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. Future studies will re-investigate alternative delivery vehicles for the bioactive molecules along with a more rigid, compressive resistant nerve guide.

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. A primate study to repair the median nerve with a longer gap using NG_{mt} was initiated about a year ago. The primate studies are summarized below.

Two groups of primate are being evaluated at Duke University. One group is to repair a 2cm median nerve gap with NG_{mt} and the other is to repair a 5cm median nerve gap with NG_{mt}. The control group is a sural nerve autograft. Electrophysiological and histomorphometrical methods will be applied to both groups and only behavioral methods will be applied to group one to evaluate the return of function. After several iterations over the past four years, 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 was at a point suitable for conducting behavioral studies. The electrophysiological studies in terms of CMAP and CSAP (compound sensory action potential) will be monitored over time to corroborate the behavioral studies. Due to recent strict regulatory enforcement of conducting large animal studies, it has taken much time to move the primate study forward. In fact it has taken several months from the Duke IACUC committee to establish compliance requirements for the investigator to follow. Thus, the progress of the primate study has moved at a very slow pace.

ISSUES

Over the past five years, we have encountered two issues that are worth considering when working with a partnership.

1) Relocation of the co-investigator: In this case one of our co-investigators, Dr. Frank Liuzzi, moved from Eastern Virginia Medical School (EVMS) to the University of South Florida (USF). His relocation has caused a delay in the rodent studies and loss of expertise that was initially planned for the project at EVMS. As a result the initial data collected were inconsistent and not useable.
2) Behavioral study using primate model: The initial design of the gripping device did not take the learning behavior of primates into account. The primates circumvented the grip design to obtain the reward upon completion of the task. A behavior much like a human being. It has taken several years to develop the final device design which can accurately isolate the grip force vectors. This coupled with recent IACUC changes of the regulatory requirements for the large animal model studies at Duke has resulted in a significant delay in the study. The resources initially allocated for the primate study were diverted to cover these delays. We were not able to complete the primate study as originally defined within the grant period and with the remaining funds.

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PROJECT TITLE: Plant Viruses as Platforms for Biomaterials

PARTNERS' NAMES AND AFFILIATIONS:

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

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

During the last year our efforts in the area of nano-medicine have had the highest priority. The Scripps group developed a cowpea mosaic virus (CPMV)-based scaffold for targeting and intracellular delivery of therapeutic compounds to tumor vasculature. The 30nm nanoparticles functionalized with vascular endothelial growth factor receptor (VEGFR1) receptor homing peptides were designed to encapsulate doxorubicin (DOX) and release at the target site. The receptor-targeted CPMV nanoparticles containing DOX were stable, bound specifically to VEGFR1 expressing cells, and induced 30% more cytotoxicity in MDA MB-231 and HUVEC cell lines than the free drug at equivalent concentrations in vitro. The DOX-loaded CPMV particles were internalized in MDA-MB-231 adenocarcinoma cells, and the intracellularly-released DOX was found to localize exclusively in the cell nucleus as determined by confocal microscopy. Intravenous injections in the tail vein of SCID mice bearing human tumor xenografts showed that the targeted virus particles localizing to the tumor tissue, specifically with the tumor endothelium. Together these studies show that CPMV nanoparticles are effective containers for delivering localized, therapeutic concentrations of DOX to tumor cells. MG Finn at Scripps has explored the use of hepatitis B virus cores and Q-beta virus-like particle cages and was able to generate expressed particles with non-natural amino acids. These allowed highly specific attachments of modified proteins using the unique side chain chemistry of this amino acid. Mark Young at Montana State University

(MSU) has targeted cowpea chlorotic mottle virus (CCMV) to CD4 positive cells with monoclonal anti-CD4 antibodies attached to the particles. He has also shown that antigenic response to these particles in mice is not a significant problem. Trevor Douglas at MSU has been able to enhance Gd binding to CCMV more than 200-fold by site-specific mutations in the CCMV capsid protein. This has dramatically increased its usefulness as an imaging agent and collaborators at NIH are exploring its properties. The release of the VLP-based pappiloma virus vaccine emphasizes the role of in vitro assembly for therapeutic virus applications. Zlotnick at Oklahoma State University has developed methods for elucidating mechanistic details of in vitro assembly and has proven the practical importance of understanding and altering polymorphic particle formation by considering these mechanisms. Doerschuk at Purdue University has now produced efficient computer code for generating maximum likelihood models of cryoEM reconstructed density. These reconstructions use a unique procedure that has great promise for dealing with heterogeneity in particle assemblies and generating initial models for particles with unknown structures.

ISSUES

As discussed last year, 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. During the last year we did studies requiring mice with implanted tumors. The costs for such work exceeded the budgets for these groups, as the original budgets were geared more for chemistry. As a result we needed to use institutional funds for this work and those funds will not be available during the next year. 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: Intelligent Systems for Detection of Aging Changes

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GRANTING NIH INSTITUTE/CENTER: National Institute on Aging (NIA)

ABSTRACT

Our rapidly aging population will result in an increasing number of people at risk for loss of independence through dementia, frailty and other syndromes of aging. Evolving sensor and other technologies now provide a means of early detection and intervention minimizing morbidity and cost. We hypothesize that integrated, continuous and unobtrusive home monitoring of activity (motor and cognitive) can detect transitional or early signal events important for maintaining cognitive and physical health. To test our hypothesis and to further develop the resulting new clinical paradigm, lead institution Oregon Health & Science University (OHSU) will establish a novel Bioengineering Research Partnership (BRP) including OHSU's Layton Aging and Alzheimer's Disease Center, Biomedical Engineering Department, and Roybal Center, and industry partners Spry Learning, Elite Care, Pultronics, HomeFree, General Electric, Hewlett Packard and Intel. The overall effort will be led by Jeffrey Kaye of the OHSU Layton and Roybal Centers, along with bioengineering Lead Investigators Michael Pavel and Tamara Hayes. Lead Investigator, Eric Dishman from Intel will provide technology industry expertise and integration in concert with leaders from the other partners. This BRP will be dedicated to developing and testing in real world environments unobtrusive intelligent systems for integrating

activities and clinical status and ultimately providing the key feedback necessary for instituting appropriate health maintenance, and illness prevention or intervention strategies. Thus our specific aims are to: 1) Determine if continuous, unobtrusive monitoring of motor and cognitive activities detects incident cognitive decline in seniors living in typical community settings; 2) Develop novel algorithms and assessment techniques for detecting motor and cognitive change in these community settings and in the context of the ongoing BRP, to test evolving sensor technology; and 3) Identify the monitoring needs of, and optimal communication channels, for lay individuals and health care professionals. As a result of this research this BRP will: establish a community living laboratory of homes outfitted with integrated sensing systems to determine early cognitive decline and identify the earliest points of cognitive change using this methodology; identify the optimal predictors and data fusion that will result in early detection; establish a fast track system for unobtrusively field-testing new sensor systems while an ongoing longitudinal study is conducted; and create a shared resource of data, expertise and community attitudes about the conduct and application of these continuous assessment techniques for future proactive application in health care.

STATUS OF RESEARCH AND PARTNERSHIP

Our grant was just awarded April 19, 2006. We have begun to scale-up our research, hiring necessary research personnel and holding regular meetings to organize the necessary research teams to address the critical issues and areas needed for successful startup and implementation of the main study. Workgroups include recruitment, clinical assessments, platforms (including hardware, broadband, tech support), algorithms, subject and staff training, retention and engagement and Mini-Living Lab development**.** Pilot work has been ongoing within the context of these workgroups addressing many issues ranging from honing of subject location methodologies to software development for tracking the in-home activity and reporting in real-time, ongoing data to the research staff. Enrollment for the Mini-Living Lab pilot is scheduled for September 1 with enrollment of up to 300 seniors living in their homes beginning February 2007.

ISSUES

Our major challenge at this point has been reorganizing to compensate for our initial 20% cut to the project budget due to current NIH budget constraints.

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PROJECT TITLE: Systems Engineering of Pheresis Intervention for Sepsis (SEPsIS).

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Steve Chang, MBA. Immunetrics Inc.

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

ABSTRACT

Severe sepsis (acute onset organ failure in the setting of infection) is a major health problem that kills nearly 250,000 Americans each year and costs billions of dollars. Available therapies for sepsis, including those recently approved, are suboptimal and new therapies are urgently needed. However, the complexities of the inflammatory response network and the high cost of clinical trials, particularly in the critically ill, renders the traditional drug/device development paradigm obsolete. We are developing and tested an extracorporeal blood purification device for treatment of sepsis based on hemoadsorption and using a mathematical model of sepsis.

STATUS OF RESEARCH AND PARTNERSHIP

So far in year one of our project we have successfully modified the sorbent used in our device and have characterized the adsorption properties using a variety of cytokines. Using these data we have successfully developed an initial mathematical model to describe the removal of cytokines in a hemoadsorption device that accounts for cytokine diffusion and adsorption within the beads. This model is now being integrated into a whole organism (rat) sepsis models to provide predictions leading to experiments in intact animals (E coli fibrin clot and cecal puncture models) to be conducted early in year 2 of the proposal.

ISSUES

Aside from delays in start up related to hiring new personnel, the partnership is proceeding as planned.

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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 (NIA)

ABSTRACT

We continue with our objective to define the role of iron metabolism aberrations in the pathogenesis of Alzheimer's Disease (AD). We are continuing to follow a cohort of carefully selected elderly control and mildly cognitively impaired (MCI) subjects with sequential psychometric studies, special MRI and magnetic resonance spectroscopy (MRS) brain images that have been specifically designed to evaluate iron content as well as peripheral blood studies that relate to cellular iron metabolism. The special brain MRI studies (known as SWI - susceptibility weighted imaging) continue to be validated by direct biochemical assays as well as SWI assays of human brains that are afflicted with amyloid angiopathy. The comprehensive genomic studies have been completed with Dr. Keith Coon of Translational Genomics of Tempe Arizona and a paper is in press on this subject.

STATUS OF RESEARCH AND PARTNERSHIP

Subject enrollment and sequential psychometric studies: Over the past 41 months we have screened over 1300 individuals and recruited 28 controls and 76 MCI participants to be studied for iron perturbations with our novel noninvasive techniques. There has been attrition in our MCI subjects; e.g. secondary to electrode implantation for cardiac difficulties prohibiting MRIs, development of malignancies, and inability to continue with the investigations because of travel difficulties. Since these cases have been replaced by further selections we have met our target for recruitment and our subject documentation is supervised by both a biostatistician and a clinical psychologist. All of our data is placed in a centralized computerized database (Access). Our control group has remained cognitively stable over the 41 month period whereas significant comorbidity and cognitive decline have been noted in the MCI cohort. Fourteen MCI cases have become demented passing on into the Alzheimer's stage with confirmed sequential hippocampal volume loss. The SWI imaging has given a quantitative and sensitive phase measure of regional brain iron content but more importantly has demonstrated microhemorrhages in 6 of the 14 progressively dementing MCI cases. This new observation with a type of MR sequencing that is more sensitive than the standard gradient echo (GE technique) is recognized for the importance of microhemorrhages and microbleeds in the pathogenesis of ongoing dementia. In addition we have conducted FLAIR studies which has enabled estimates of white matter hyperdensities indicative of small vessel disease which correlates with the microhemorrhages and microbleeds.

Special SWI imaging and Spectroscopy: A total of 255 studies have been conducted on the study cohort (MCI and control) over the past 41 months. Many cases have had sequential studies including one case that turned out to be multiple systemic atrophy demonstrating marked iron deposition in the nigral pallidal pathway which is being studied concurrently with Dr. Ronald Petersen of the Mayo Clinic. Arrangements have been made for autopsy in this case with the family in view of the remarkable findings on SWI imaging in terms of

iron deposition in the nigro-pallidal the fiber tracks. Spectroscopy has been performed concurrently with imaging and consisting of a direct metabolite level measurement of choline (Cho), creatinine (Cr), N-acetyl-neuraminic acid (NAA), myoanisitol (Mi), glutamine/glutamate (Glx) in the posterior cingulate gyruses. The decreases in Cr, Glx, and NAA have been observed in the MCI and progressive MCI cases and have been compared to age matched controls.

Blood studies: A total of 418 blood draws have been performed on our cohort MCI population and elderly controls. We have been focusing on measuring levels of pro-inflammatory cytokines in MCI compared to healthy age matched controls. Our data indicates that the alterations in the cytokine productions by peripheral blood mononuclear cells can be detected early in MCI and that a shift towards the pro-inflammatory phenotype precedes the development of AD. Interestingly proinflammatory cytokine production seems to peak in MCI patients and then returns to control or below control levels once they progress to AD. This is in agreement with findings that intrathecal inflammation precedes the development of AD that gives support to the hypothesis that inflammation is an initiating factor rather than a late consequence of the disease. A manuscript has been submitted. Another blood study that is being conducted has been a study of brain blood iron perturbations in the pathogenesis of AD by determining changes in level and function of iron regulatory protein 2 (IRP-2) in peripheral blood. This study has been done by taking whole blood RNA and serum samples obtained from both control and the amnestic multiple-domain MCI participants and utilizing Quantitative Realtime PCR to investigate expression levels of IRP-2. In order to assess the functionality of IRP-2 the expression of three alternative splice forms of the divalent metal transporter 1 (DMT1) was also evaluated. Through nested PCR and DNA sequencing we were able to identify several alternative splicing variants of IRP-2 whose function and possible correlation to AD is currently being assessed in relation to participants' clinical course. To our knowledge this is the first report on the identification of IRP-2 splice variants in experimental animal or humans. The significance of these variants is under current investigation. Another aspect of our blood studies is the attempt to discover a serum biomarker for early stage AD. We have partnered with George Mason University and will provide them with Alzheimer's disease serum and brain tissue (UCLA) to attempt biomarker discovery utilizing advanced proteomic techniques.

Genomic studies: We have reported on our `genomic studies. The paper "Preliminary demonstration of an allelic association of the *IREB2* gene with Alzheimer's Disease" is in press in the *J of Alzheimer's Disease*.

Knockout mice SWI imaging and micro dissection: These studies have been completed. A paper is in preparation. We are currently attempting to validate the presence of iron and correlate with the MR SWI signals.

The major significant finding of our study so far has been the fact that in the MCI population that is showing rapid dementia we have found with our SWI technology the evidence of microbleeds in 6 of the 14 dementing patients. The presence of these small microbleeds are black dots on phase measurements and is significant in that they seem to be increasing as the dementia and cognitive status increases implying that these microbleeds are not evident on the standard gradient echo imaging technique that is currently being used. This is a major development and may lead to an early diagnosis of amyloid angiopathy. We have evidence of significant differences in the proteomics of blood, particularly with LDL receptor protein 4 found only in the suspected amyloid angiopathy cases. This study is still in progress with Drs. Liotta and Vinters.

We plan to extend our recruitment as advised by our consultant Dr. Ronald Petersen in view of the significant findings of a possible way to diagnose amyloid angiopathy premorbidly. The proteomic study and the SWI technology offer a new advance in studying the pathogenesis of the dementia and the vascular aspect of dementia that occurs with AD.

ISSUES

None.

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PROJECT TITLE: Manipulating Neural Tissue With Ultrashort Laser Pulses

PARTNERS' NAMES AND AFFILIATIONS:

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

ABSTRACT

The proposed work involves a conjunction of discovery-directed studies and technological development in support of those studies. The discovery-directed goals address the large-scale organization of neuronal and vascular anatomy in cortex and the associated blood flow dynamics. A unique aspect of these studies is the use of highenergy, *i.e.*, > 0.1 microjoule, and ultrashort, *i.e.*, ~ 100 femtoseconds, laser pulses to manipulate tissue. These manipulations allow us to access all depths of tissue for histological analysis and, for the case of hemodynamics, allow us to perturb flow in targeted arterioles, venules and capillaries and thus study issues such as vascular perfusion in real time. The work is centered at UCSD The technology-directed goals address three issues: (1) The development of long working distance, high numerical aperture objectives to facilitate the in vivo ablation of tissue as well as imaging across large regions of cortex. Their design will minimize spherical aberrations and temporal distortions. (2) The development of fast scan systems, using temporally multiplexed beams, to achieve throughput rates limited only by the lifetime of the fluorescent indicator. This will allow near GHz acquisition rates. (3) Algorithms and standards for the automated visualization and quantification of neuronal and vascular architectonics. Work on items 1 and 2 is centered in Colorado, while item 3 is the purview of our SAIC partners.

STATUS OF RESEARCH AND PARTNERSHIP

We continue to focus and make program on the above goals and partnership remains fruitful and strong. Seven manuscripts, 5 with original data and 2 reviews, have been published.

Our current scientific focus is on two issues:

Large-scale distribution of subsurface vasculature and neurons. We are use our all-optical histology to automatically image, in three dimensions and with micrometer resolution, labeled DNA a means to map the distribution of all cell nuclei, labeled neuronal nuclear protein as means to map the distribution of neuronal nuclei, and labeled vasculature. These yield the cell

count density and the microvasculature volume fraction density versus distance into the mouse cortex.

Temporal and spatial dynamics of blood reperfusion in the vicinity of microclots. We have developed an optical-based stroke model, using ultrashort pulses to target microvessels below the pia and linear absorption to target surface vessels, to produce microclots and visualize flow dynamics in and around the clotted vessel. This model provides a unique means to quantify vascular perfusion, collateral blood flow, and other issues related to stroke formation. Our results point to a fail-safe mechanism of flow in the higher interconnected surface vessels but a bottleneck to flow in the penetrating arterioles that feed the underlying microvascular bed.

ISSUES

Our realization of a Biomedical Research Partnership has promoted the most productive collaborative effort of my academic career.

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PROJECT TITLE: Analyses of InSitu and Explanted Surgical Implant Device

PARTNERS' NAMES AND AFFILIATIONS:

Investigator Charles Patrick (Tissue Procurement: [Alabama Organ Center, Alabama Tissue Center, Regeneration Technologies Incorporated (AOC/ATC/RTI)]

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

ABSTRACT

Recommendations from governmental and professional society based conferences on "improvements in health care through analyses of explanted surgical implant devices," and specifically the NIH-BECON initiatives, has afforded a new opportunity for multidisciplinary research to study this critical problem through the NIH-BRP mechanism. We proposed a unique approach to the study of cadaveric in situ implants for direct comparison with changes noted from explanted orthopaedic devices to demonstrate the feasibility and value of these type studies with an overall intent to answer basic questions intended to enhance device and treatment longevities. This new approach minimizes many of the limitations that have existed for device retrieval and analysis programs. Bioengineers, physicists, physicians, statisticians, records personnel and students now interact on a single university campus to more fully investigate relationships among host, surgical technology and device properties as they relate to recognized biocompatibility issues that could compromise longevities for younger patients. The focus is on tissue and host responses to debris generated from articulation and fixation of bone interfaces for total hip and knee prostheses. This partnership with organ and tissue recovery facilities is used to develop and evaluate: a new infrastructure; protocols and standard operating procedures; and participation in professional society and standard organizations. Activities include: management and review by directors of the partnership through intra-and extra-mural committees; use of existing cadaver processing facilities of the partnership through intra- and extra-mural committees; use of existing cadaver processing facilities and core laboratories for obtaining, processing and analyzing specimens; and joint meetings and studies based on research hypotheses and consensus protocols. Sequential activities focus on feasibility and value at one center for infrastructure, information and assessment in the first years intended to enhance treatment and enhanced quality and longevity of life in an active and aging population treated with prosthetic devices.

The specific aims for this proposed program is focused on demonstrating feasibility and value of meticulously examining in situ and explant devices by biological, physical, mechanical and chemical means and placing this in context with host biocompatibility characteristics as follows.

1. To refocus the existing program on analyses of explanted devices and tissues and develop the infrastructure for identifying, removing and studying in situ (cadaveric) orthopaedic hip and knee replacement systems.

2. To develop and test Standard Operating Procedures (SOP's) for evaluating cadaveric specimens in conformance with regulatory (IRB and HIPAA) guidelines and to determine numbers and types within the collaborating (partner) organ and tissue donation programs.

3. To conduct multidisciplinary studies of statistical significance with in situ and explanted orthopaedic devices to answer critical questions affecting in vivo longevity and to provide a basis for investigator initiated RO1 proposals specific to device related questions.

4. To engage in monthly, quarterly and annually timed meetings with intramural and extramural advisory committees to assess progress.

5. To continually evaluate the feasibility and value of the program and to assess the possibility of expanding it as originally proposed.

STATUS OF RESEARCH AND PARTNERSHIP

Recommendations to enhance longer term total joint articulation through reduction in fretting and wear phenomena; increased attachment percentage and strength through surface modifications of biomaterials; and increased quality of function and longevity for longer-term in-dwelling prostheses and devices.

During the year 2005-2006 we collected about 400 additional device specimens; have held monthly meetings, and have set-up the system for overall data entry and recovery.

One special focus area is metallic ion transfer and local and systemic interactions with cells, tissues and organs for longer-term (decades) metallic articulation total joint arthroplasties. Issues being raised about hypersensitivity and carcinogenicity might be confirmed (or not) from appropriate cadaveric sampling including in depth device and pathology oriented investigations.

Other on-going activities on component fixation to bone by surface modifications (nanostructured calcium phosphate compounds and/or nanostructured diamond) could also come into consideration as associated clinical trials progress.

We believe that the tissue interface information could serve as a platform of science and technology for new tissue engineered medical products (TEMPS) and biodegradables for regenerative medicine.

We are currently seeking additional sources for selected types of cadaveric implants through other (national) body donation programs; and unique identification of donors through collaborations with orthopaedic surgery professionals

ISSUES

A central concern during 2005-2006 was the unanticipated public news about possible illegal processing and transferring of human body parts from one location (Northeast, USA) to legal and approved facilities for organ and tissue replacements. Because of unknowns over the short term, cadaveric specimen activities were delayed. Efforts were refocused on explanted devices and expanded and carefully controlled standard operating procedures and protocols for cadaveric specimens.

The issue of identification for illegal transfers has been completed (no BRP transfers) and cadaveric specimens are now being identified, removed en bloc and transferred. One benefit of this delay was the identification and expansion of sources, which will be important to future (years 2-4) aspects of this BRP program.

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PROJECT TITLE: Methods & Noninvasive PK Study to Improve Iontophoresis

PARTNERS' NAMES AND AFFILIATIONS:

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Paul S. Bernstein (Department of Ophthalmology and Visual Sciences, Moran Eye Center, University of Utah)

GRANTING NIH INSTITUTE/CENTER: National Eye Institute (NEI)

ABSTRACT

The existing methods to treat posterior eye diseases are intravitreal and periocular injections, surgical implants, and systemic drug administration. Systemic administration for the treatments of posterior eye diseases is usually not preferred because of the systemic toxicity encountered. Intravitreal and periocular injections and surgical implants are more effective treatments compared with systemic drug delivery, but repeated injections and surgery can cause side effects such as severe pain, intraocular bleeding, increased chances for infection, and the possibility of retinal detachment. Ocular injections and surgical implantation also involve high health care cost because of the participation of experienced ophthalmologists in carrying out the treatments. An effective low-cost robust noninvasive drug delivery system for the treatments of posterior eye diseases has yet to be developed. There is also a lack of human pharmacokinetic data of ocular drug delivery in general. The development of a noninvasive approach to study ocular drug pharmacokinetics in the posterior of the eye will be beneficial in ocular drug delivery research. Ocular iontophoresis is a noninvasive method and has the potential for drug delivery to the posterior of the eye. The main objectives of the present project are (a) to characterize the transport properties of ocular iontophoresis and develop a more effective iontophoresis method for the treatment of posterior eye disease and (b) to develop a magnetic resonance imaging (MRI) technique for ocular pharmacokinetic studies and perform these studies following ocular drug delivery such as iontophoresis.

STATUS OF RESEARCH AND PARTNERSHIP

This research has been funded for 4 months. The members in the partnership are working well together. The two major components of the project up to this point have been the study and development of an iontophoresis system that is more robust than the existing device and the development of F-19 MRS and MRI for noninvasive pharmacokinetic studies.

In the iontophoresis device development, the objective was to characterize ion-exchange membrane-enhanced transscleral iontophoretic transport, in which an ion-exchange membrane system was utilized to enhance transscleral iontophoretic delivery, so lower electric current and shorter duration of iontophoresis application (more effective ocular iontophoresis) can be used. In order to understand the mechanisms of enhanced ocular iontophoretic delivery and to improve this method, the physical properties of the ion-exchange membrane and the sclera were characterized. Transscleral transport experiments with the ion-exchange membrane were conducted with excised rabbit sclera in vitro. The contribution of electroosmosis to electrotransport during transscleral iontophoresis was assessed. So far, the ion-exchange membrane provides a three-fold steady-state transscleral flux enhancement over conventional

transscleral iontophoresis. To further investigate the mechanisms of ion-exchange membrane-enhanced iontophoretic transport, the iontophoretic transport behavior across multiple membranes of different barrier properties was studied. Computer model simulations were performed to study the contribution of diffusion, electromigration, and electroosmosis across the system of multiple membranes. The initial results of the model analyses suggest significant contribution due to diffusion in iontophoretic transport across a multiple membrane system such as the system of ion-exchange membrane-enhanced transport.

In the pharmacokinetic study and development, the first phase of the study was to modify a clinical MRI system for F-19 magnetic resonance spectroscopy (MRS) in future animal and human clinical noninvasive pharmacokinetic studies. NMR development was performed on a clinical MR imaging system (3T Trio) from Siemens Medical Solution (Erlangen, Germany). The system is equipped with multi-nuclei NMR capability, which includes MR spectroscopy and MR imaging. Necessary hardware, pulse-sequences, and processing program were developed for F-19 MRS, which provides (1) active T/R switching using PIN diode switching and passive T/R switching by utilizing $\lambda/4$ (quarter wavelength) coaxial cable and crossed diode; (2) preamplifier: 3-200 MHz, 30 dB gain, 1.0 dB noise figure from Miteq; (3) F-19 MR imaging coil: 1.5 inch transmit-receive surface coil and single-turn solenoid coil with 2" diameter and 6" length; (4) two pulse sequences were modified for MR imaging of F-19 MRI/MRS: gre_F: gradient-echo imaging sequence and fid_x: FID sequence. The modified MR imaging system was first tested with perflurocarbon (PFC) using active T/R switch and F-19 STS volume coil. Satisfactory F-19 MR images were obtained. Then, F-19 spectra were acquired using the 2" surface coil and fid_F sequence for dexamethasone phosphate after intravitreal injection of dexamethasone phosphate in rabbit eye in vitro. Satisfactory F-19 MR spectra were obtained.

In addition, preliminary MRI studies using contrast agents were conducted to investigate the route of penetration, ocular barriers, and the factors affecting transscleral iontophoresis. The delivery and distribution of the model permeants (the contrast agents) into the eye during and after iontophoresis were determined with MRI. It was found that transscleral iontophoretic delivery was related to the position and duration of iontophoresis application, and electrode placement was an important factor. In addition to the effect of electrophoresis, electric field-induced tissue alteration was observed, and the importance of clearance and the vasculature barrier in ocular delivery was demonstrated. These preliminary results provide helpful experience before the F-19 animal and human studies and new insights into ocular delivery pharmacokinetics.

ISSUES

The PI moved from the University of Utah to the University of Cincinnati earlier this year. The grant transfer application (CGI application) was submitted last December and the funding was temporally stopped after that. For some reason, the grant transfer process was delayed for six months. Last month, a new grant management specialist was assigned to the case and the CGI application finally got moving. In the past six months, we have continued the BRP project using the PI startup funding at Cincinnati and other funding at Utah.

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PROJECT TITLE: Multi-modality Biomedical Imaging of Cancer/tumor hypoxia

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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI)

ABSTRACT

Several lines of investigation have indicated that tumor hypoxia has a negative impact on clinical response. The fundamental hypothesis driving our research program is that non-invasive imaging of tumor hypoxia will lead to improved management of patients with solid tumors. However these images may be influenced by factors other than tumor hypoxia leading to possible ambiguity and the potential for misinterpretation.
Our approach has been to develop methodologies that enable verification of non-invasive images of tumor hypoxia. The two key themes are:
1 To establish techniques of visualizing and quantifying tumor hypoxia based on positron emission tomography (PET), magnetic resonance imaging and spectroscopy (MRI/S), digital autoradiography (DAR), immunofluorescence microscopy and oxygen probe measurements.
2. To establish methodologies of integrating the information from these diverse sources into a coherent "big picture" based on the use of a common 3D spatial coordinate system for all measurements together with image/data registration and analysis of statistical correlation.

STATUS OF RESEARCH AND PARTNERSHIP

Correlative studies of tumor hypoxia imaging

We have performed multiple studies to examine the correspondence between the spatial distribution of PET hypoxia tracers and alternative indicators of tumor hypoxia. In addition to other models discussed in previous reports we have used the human colorectal adenocarcinoma cell line HT29 growing as xenografts in nude mice and rats. The HT29 system expresses a high level of the hypoxia-regulated protein carbonic anhydrase 9 (CA9) that provides an additional basis for verification of tracer distribution. In unperturbed tumors the spatial distribution of CA9 is similar to that of the 2-nitroimidazole pimonidazole administered shortly before animal sacrifice. However the relatively long ($T_{½}$ ~ 96hr) protein lifetime of CA9 means that its expression pattern is more representative of hypoxic history that the relatively instantaneous visualization provided by pimonidazole. We have used these diverse properties to study perturbations of tumor hypoxia induced by carbogen (95% O_2: 5% CO_2) breathing, hydralazine administration and mild hyperthermia. CA9 expression remains essentially constant irrespective of the perturbation but pimonidazole uptake is perturbation-dependent. We have quantified CA9- and pimonidazole-positive fractions and can compare these to DAR quantification of the uptake and distribution of dual PET tracers where one tracer is administered before and one after perturbation. This enables us to assess the ability of PET hypoxia tracers to track changes in tumor hypoxia.

Hypoxia-driven reporter systems

In previous reports we described the generation and use of a hypoxia-reporter system based on a retrovirally transduced variant of the Dunning R3327-AT cell line containing a HSV1-tk/enhanced Green Fluorescent Protein (TKeGFP) fusion gene under the regulation of an artificial hypoxia responsive enhancer/promoter. We are working in collaboration with Dr Gloria Li of MSKCC to generate further hypoxia reporter systems based on the HT29 cell line and candidate clones are currently being tested. Such models enable imaging of reporter gene expression using appropriate tracers by PET and DAR.

Pilot studies with TF-MISO

2-Nitro-alpha-[(2,2,2-trifluoroethoxy)methyl]-imidazole-1-ethanol (TF-MISO) was investigated as a potential non-invasive marker of tissue oxygen levels in tumors using ^{19}F MRS and ^{19}F chemical shift imaging (CSI). *In-vitro* data on tumor cells incubated under different oxygen conditions showed that TF-MISO preferentially accumulates in

cells incubated under hypoxic conditions. *In-vivo* data were obtained using the hypoxic murine MCa breast and rat prostate R3327-AT tumor models implanted in nude mice. Detection of intra-tumor ^{19}F signal from TF-MISO was performed using MRS for up to 8-10 hours following a 75 mg/kg intravenous injection. Localized distribution of the compound was imaged at a resolution (~3×3×3 mm^3) using slice selective 2D-CSI six hours after injection. The ^{19}F MR spectral features (linewidth and chemical shift) were recorded as a function of time after injection and the results indicate that the fluorine atoms are indeed sensitive to changes in the local environment while still providing a detectable MR signal. *Ex-vivo* spectra were collected and established the visibility of the ^{19}F signal under conditions of maximum hypoxia. Late time point (> 6 hour) tumor tissue concentration, as obtained from ^{19}F MRS, suggest that TF-MISO is reduced and retained in hypoxic tumor. The feasibility of obtaining TF-MISO tumor distribution maps in a reasonable time frame was established. Based on these results, TF-MISO may have the potential to be a valid MRI hypoxia reporter for both pre-clinical hypoxia studies and hypoxia directed clinical therapy.

Clinical implementation of the tumor hypoxia PET tracer ^{124}I-IAZGP

Our animal experiments discussed in previous reports led us to identify the 2-nitroimidazole compound iodo-azomycin-galactopyranoside labeled with the positron-emitting radionuclide ^{124}I (^{124}I-IAZGP) as a promising candidate for tumor hypoxia imaging. We now have an FDA-approved IND for this agent and have begun clinical imaging studies. In the first instance the focus of these clinical studies is to establish the safety and define biodistribution, pharmacokinetics and radiation dosimetry. We also anticipate that these preliminary studies will establish the optimal time post-injection for tumor hypoxia imaging. So far three patients have been administered ^{124}I-IAZGP and whole body PET images produced up to 48 hours post-administration.

Implementation of Robot system

In collaboration with the Bio-Engineering Group at John Hopkins University we have developed a robotic system with the capability to perform image-guided procedures under computer-control. Computer-controlled motors drive the x-y (horizontal) motion of a platform (to which the same rodent bed used in the animal MRI and microPET devices is rigidly attached) and the z (vertical) motion of a micro-manipulator. Integrated application software controls device motion, display and manipulation of 3D tomographic image data and registration of the 3D co-ordinates of the robot with those of the microPET or MR images. This system builds upon our experience with the fiduciary template system described in previous reports but provides us the opportunity to probe, sample and inject at any point in the image set rather than being constrained by template location. Preliminary studies of system accuracy indicate a target registration error of ≤0.2mm however we expect to improve on this figure as our experience increases.

Compartmental Modeling-Based Parametric Imaging of Tumor Hypoxia

In collaboration with Philips Medical Systems and utilizing a beta version of their newly developed Bio-Guide™ software, we have begun to derive parametric images of tumor hypoxia by voxel-based compartmental modeling of dynamic microPET images of ^{18}F-FMISO in rat tumor xenografts. The model for FMISO comprises two compartments, a "non-specific" (i.e., non-hypoxia-related) compartment common to all tissues and a "specific" compartment found only in significantly hypoxic tissues. A blood time-activity curve (TAC), measured by region-of-interest analysis over a blood pool area (e.g., left ventricle or abdominal aorta) identified in the dynamic microPET images, provides input to the non-specific compartment; the exchange of tracer between the blood and the non-specific compartment is characterized by a "forward" rate constant k_1 and a "reverse" rate constant k_2. From the non-specific compartment, radiotracer is transferred to and irreversibly trapped in the hypoxia-specific compartment with a rate constant k_3. So far we have successfully imported dynamic microPET ^{18}F-FMISO images into the Philips Pinnacle™ platform, derived blood and tumor TACs from the dynamic ^{18}F-FMISO images, successfully fit voxel-specific TACs in tumor to the compartmental model and derived ^{18}F-FMISO parametric images wherein the value of each voxel represents the respective voxel's k_3 value. The "k3" parametric images derived thus far appear to provide higher hypoxia to non-hypoxia contrast than native ^{18}F-FMISO images. In this respect, the ^{18}F-FMISO "k3" parametric images more closely resemble "late" (i.e., ~24 hr post-injection) ^{124}I-IAZG than the "early" (i.e., ~2 hr post-injection) ^{18}F-FMISO images. These results are highly preliminary, however, and several issues (such as derivation of reliable input functions and refinement of the model topography) remain to be resolved.

ISSUES

None.

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PROJECT TITLE: BION Treatment of Neuromuscular Dysfunction

PARTNER'S NAME AND AFFILIATION

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

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 new class of generic devices that can deliver precisely metered stimulation pulses to an arbitrary number of nerve and muscle sites. 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 will design and build BION 1 implants and accessory components for testing, programming and controlling them in patients. We will investigate the safety, efficacy and clinical utility of BION™ technology for electrically activating muscles that are paralyzed and/or atrophic in order to prevent and/or treat the following conditions in stroke patients: Post Stroke Subluxation; Exercise Programs for Wrist and/or Finger Flexor Tone and Contracture Management; and, BION-induced Training post-Stroke to Enhance Recovery of Hand Function. Advances in BION technology, such as increased power efficiency, improved ASIC design and portability as well as sensor and back-telemetry capabilities for functional electrical stimulation (BION2E and BION2) will be deployed once their safety has been determined. In subsequent years, 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 BION2 technology to provide functional reanimation of paralyzed limbs using neural prosthetic control.

STATUS OF RESEARCH AND PARTNERSHIP

A major objective of pilot investigational studies is to identify design weaknesses and eliminate them before the final product is validated in a larger pivotal trial. After having implanted a total of 79 BIONs

in 35 participants in five different clinical trials since 1999, we discovered last spring that four unresponsive implants were, in fact, visibly broken on x-ray. While this is not technically an adverse event by conventional regulatory criteria, we voluntarily suspended all clinical trials and began a systematic failure analysis. One important clue was that these failures had occurred in participants with relatively deep insertions of BIONs more perpendicular than parallel to the muscle fascicles and in muscles that had been substantially strengthened as a result of aggressive and prolonged stimulation programs. In every case, the device was broken at the tantalum end of the BION 1-2 device. A manufacturing review was held within 24 hrs of inspection of radiologic confirmation of the break in the first subject (March 10, 2005). With the exception of one device that appears to have rotated 180°, none of the other broken devices appear to have migrated. No further changes in the position or the state of the four failed devices have been observed during the last 15 months. BION 1-2 package remediation has been completed, we have received clearance from the FDA to resume trials (G010068, 04/20/2006; G030147, 04/20/2006; and G040143, 05/05/2006) and are in the process of obtaining IRB approvals to re-initiate subject recruitment.

Remediation of BION Mechanical Failure: The original validation process had focused on breaking strength of the glass capsule but had not considered the effects of repetitive bending stresses on the package. 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 causes 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. We reexamined our original assumptions about maximal stress on BIONs implanted within muscle to consider also configurations in which the electrodes could be embedded in tendons that moved independently from the muscle in which the rest of the capsule was embedded. We concluded that it was possible for a physician to implant BIONs in such a way that 1.8-4.2N of shear force could be applied to each electrode by a tetanically contracting muscle. Such forces are well below the static breaking loads for the capsule but are consistent with the hypothesized stress fatigue mechanism**.** The theoretical analysis of maximal stress and the conditions of use in the current clinical trials led to a clear statement of the worst-case scenario that the BION implants needed to be designed to survive. Several methods were considered to strengthen the BION. The preferred method was to fuse the glass capsule directly to the sintered tantalum slug, eliminating the free neck of the tantalum stem. This avoided the introduction of new package materials but it did require the development and validation of a new process for melting the glass bead against the tantalum slug in a vacuum furnace so as to provide a hermetic seal. A validation test battery was devised and approved, based on the above analyses and consideration of the hazards and risks that might have been introduced by the design changes. All test devices built according to the revised design have survived >180,000 cycles of loading with the maximal theoretical forces. Longer term tests are underway to identify the ultimate limit; one sample has now survived >1,550,000 cycles and no failures have occurred.

Clinical Trials: Despite the hiatus in new implants, the clinical trials team has been busy collecting outcome measures and long-term follow-up from the 30 patients enrolled in the shoulder and hand studies. We have also conducted a number of fitting and feedback sessions with selected patients and therapists to make improvements in the reliability and usability of the external equipment with which they are provided. These data are among the determining factors in the design and development of the new BION controller.

ISSUES

None.

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PROJECT TITLE: Blind Pedestrians' Access to Complex Intersections

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

ABSTRACT

The goals of this partnership are to identify street-crossing problems experienced by pedestrians with blindness and low vision at complex, unfamiliar intersections, and to develop and test devices and strategies for enhancing intersection access for these individuals. Research projects include the study of street crossing behavior at roundabouts, the design, installation and operation of accessible pedestrian signals, the eye gaze strategies of individuals with age-related macular degeneration and glaucoma, access issues related to interactions of drivers and pedestrians, the auditory abilities of blind individuals as they relate to the street crossing task, the development and testing of a device to reduce the tendency of blind pedestrians to veer, and the development of virtual acoustic technology.

STATUS OF RESEARCH AND PARTNERSHIP

Western Michigan University, Lead Institution, Lead Investigator Richard Long

During this year, the Western Michigan engineering team completed the engineering design and fabrication work on the "Anti-veering training device" (AVTD). The testing protocol for the device was designed during this project period, and the protocol will be implemented in 2006-2007. The Western Michigan team also completed the data analysis of our investigation of in-roadway vehicle detection technology at a roundabout intersection. We completed a study in the metropolitan Baltimore area that involved the effect of geometric features of roadways (curves, hills) on blind pedestrians' abilities to detect approaching vehicles at uncontrolled crossings. Primary goals for the upcoming year include testing of effectiveness of the AVTD's and working with NCSU investigators on video-based yield and gap detection technology for use at roundabouts

Maryland School for the Blind/Johns Hopkins University, Lead Investigator Duane Geruschat

We continued our data collection on a study of roundabouts access for persons with low vision. The data collection system was pilot tested and found to be reliable and to data appropriate for the study questions being asked. We also have revised the entire approach for the collection and analysis of eye tracking data as they relate to access to information at intersections

by persons with low vision. We will collect pilot data during early fall, 2006 for studies scheduled to begin in the early spring of 2007.

North Carolina State University, Institute for Transportation Research and Education, Lead Investigator Nagui Rouphail

Our plans for 2007 are to work with the Western Michigan team to develop and test an improved yield and gap detection system that relies on video-zone detection technology. Outcome measures for system evaluation will include the safety and delay of crossing with and without the system in operation. This project is important to transportation engineers because a video-based yield detection technology, if successfully implemented, would negate the need for signalization to provide access for blind pedestrians at single lane roundabouts, thus eliminating the potential negative impact of signalization on vehicle operation. Concurrent with the technical development and behavioral studies of the yield and gap detection system, a vehicle-pedestrian modeling effort will be carried out in the upcoming year.

Vanderbilt University, Lead Investigator Dan Ashmead

During the 2005-2006 project year, we began a series of studies to relate the research literature on perception of "time-to-contact" to the pedestrian street crossing task. We created a computer graphics display with a roadway scene and realistic images of vehicles. The main purpose of our first experiment was to extend findings on time-to-contact judgments to the time ranges with which pedestrians must operate. During summer of 2006 we will extend this work to include different viewing angles, a wider range of vehicle speeds, and a range of vehicle sizes. In fall of 2006 we will extend this work to an existing immersive graphics system with a goggle display, which will update the scene based on observer movement and provide separate images to each eye to test for binocular contributions. During winter and spring of 2007 we will begin to explore the effects of visual impairment on perception of time-to-contact, in terms of both visual field restrictions and contrast sensitivity deficits. The second focus of the Vanderbilt team will be on continuing our work on auditory perception of vehicle motion paths. A series of experiments will be completed to test which kinds of perceptual information are most useful for performance of vehicle detection. In Winter/Spring of 2007 we plan to use this methodology to compare auditory motion perception in adults who are sighted vs. totally blind.

Boston College, Lead Investigators Randy Easton and Billie Louise Bentzen

Boston College researchers have continued to field-test the effects of optimized accessible pedestrian signals (APS) on the safety, wayfinding, and independence of blind pedestrians as they crossed complex unfamiliar signalized intersections in four cities (Cambridge, MA, San Diego, Charlotte, and Portland, OR). The investigators compared pre- and post-installation performance of blind participants in a variety of street-crossing tasks. As the research progressed, refinements in the technology and installation were made in each city based on results of previous field testing. In the upcoming year, the redesigned APS will be compared to an earlier prototype APS in a third round of testing in Charlotte. A third round of testing also will be conducted in Portland to enable the comparison of two different technologies for providing safety and wayfinding information for blind pedestrians at complex intersections.

ISSUES

None.

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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 (NIBIB)

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 fifth year research goals described in the proposal. The animal testing of the second generation of the clot extraction system has been completed. We are currently in animal studies with the stent for both functional testing and long-term biocompatibility. 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 no inflammation response on the first two animals after one month of stent implantation. 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. By the end of the fifth year we expect to have 20+ patent applications filed and 20 peer-reviewed publications. Finally, we are in licensing discussions with a major medical device company.

The project has led to five other grants including two SBIR Phase I awards for foam based devices (listed under other support for the PI), a seed grant to develop analytical tools for measuring the impact of the SMP devices on the fluid and thermal transport of the vessels (LLNL seed, $240k/yr direct for three years, Maitland PI), a grant to study the in vivo functionality of SMP foams for treating saccular aneurysms ($200k total, Hartman, PI), and a LLNL seed award for developing new materials (Tom Wilson is PI with Jane Bearinger). Also of significance is the award of four separate capital equipment grants. The progress to date motivated DOE OBER, the NSF Center for Biophotonics Science and Technology, the Medical Physics and Biophysics Division at LLNL and the LLNL Laboratory Directed Research Office to award year-end money approximately totaling $565k.

ISSSUES

There are no significant issues on the project.

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

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Mr. Larry Nielsen (Philips Medical Systems, Inc.)

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

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 and continuous assessment of the clinical state of each patient. However, 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 knowledge-based reasoning to design a new generation of monitoring systems to track 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. The specific goals of our research effort are:

1. *Database development and annotation*: Collect and annotate a comprehensive new database from ICU patients to support research in patient monitoring. The records will contain detailed physiological and clinical data along with UMLS-coded physician annotations of hemodynamically significant events.

2. *Database de-identification and distribution:* De-identify the database and make it available to the research community via our NCRR-funded Research Resource for Complex Physiological Signals (http://www.physionet.org).

3. *Development of an Advanced Monitoring System:* Develop innovative algorithms and clinician interfaces to assist in the annotation process and to form a prototype advanced monitoring system.

3. *Testing of an Advanced Monitoring System:* Evaluate the new monitoring concepts beginning in the laboratory utilizing the new database. Later, evaluate industry-constructed monitoring system prototypes in clinical settings at Beth Israel Deaconess Medical Center.

STATUS OF RESEARCH AND PARTNERSHIP

Database - The database (MIMIC II) has grown enormously and currently contains approximately 17,000 ICU patient records containing multi-parameter physiologic measurement trends, nursing progress notes, medication records and laboratory data; 2,500 of these records also include continuous physiologic waveforms. Advanced software from Philips has been installed at the hospital that permits us to collect more channels of physiologic data at increased resolution from each patient. De-identification software has been developed and used successfully on text portions of the database, and as a result this data is available to our entire multi-institutional research team. Annotation of the database is progressing slowly. We recently released more than 60 gigabytes of waveform data to the research community via www.physionet.org/physiobank/database/mimic2db/ .

Data Analysis - Innovative and sophisticated algorithms and clinician interfaces are being developed that will assist in annotating the database, and will lead to a prototype advanced monitoring system. New developments include: a sophisticated search engine to identify times of occurrence of pre-defined patterns in multi-parameter physiologic time series; signal quality indices to identify when waveform data is trustworthy; software to estimate cardiac output trends from arterial BP waveforms; and new techniques to drastically reduce the frequency of false BP and arrhythmia alarms.

Modeling - Model-based signal processing methodologies are being used to integrate multivariate clinical data streams into a comprehensive framework that facilitates and enhances monitoring, alarm generation, clinical analysis and intervention planning in the ICU. Efforts are under way in developing and evaluating cycle-averaged models of cardiovascular dynamics, probabilistic models using Bayesian networks, and in investigating the potential value of spectral estimation methods in relating heart rate variability to mortality using the MIMIC II database.

Reasoning – Two studies are focused on dealing with textual data, in the context of locating information that must be removed to protect patient privacy, and information that must be recognized to identify diagnoses and interventions. Both efforts have yielded algorithms that perform well. We have very good de-identification software, and extraction of diagnoses from discharge summaries is in routine use in our annotation workstation. Other efforts are focused on using available data to predict the clinical course of ICU patients.

The partnership - The partnership continues to function smoothly, and the three institutions (university, industry, and hospital) are critical to making this research feasible. 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 Beth Israel Deaconess Medical Center supports our IRB-approved data collection in the ICUs and from hospital data archives. We continue to collaborate with BIDMC clinicians in data-mining of our ICU database to address important clinical questions such as: a) what is the relationship between ventilator settings and the development of ARDS, and b) is it possible to develop an early warning detector of impending septic shock? 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, MA and in Briarcliff Manor, NY participate with us as research colleagues. We have an outstanding external Advisory Board which meets with us annually.

ISSUES

We were not able to expand our data collection effort to the University of Massachusetts Medical Center because the key collaborating clinician left the institution. Technical problems halted our data collection effort at BIDMC for almost a year. Fortunately, Philips engineers were successful in solving the problem with new software and hardware, and data collection has resumed.

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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 (NIBIB)

ABSTRACT

Purpose The purpose of 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, fluorescent staining and optical imaging, analysis of the release of dipicolinic acid, and phase contrast microscopy 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 is not sufficient to achieve 6-log reduction of spores. We have also confirmed that CO_2 + water, CO_2+L-alanine (a germinant), CO_2+lysozyme (an enzyme), CO_2+ethanol, and CO_2+isopropanol are not efficient in killing bacterial spores. However, parts per million (ppm) quantities of 30% aqueous hydrogen peroxide

(H_2O_2) solution in CO_2 does result in 6-log reduction of *B. atrophaeus*, *G. stearothermophilus*, *B. pumilus*, and *B. anthracis* spores. TEM imaging indicates disruption of the glycoprotein exosporium. Spores treated with CO_2-based mixtures release small quantities of dipicolinic acid, and we have identified damage of spore permeability barrier with the help of a *Bac*Light fluorescent assay. We also confirmed with phase contrast microscopy that the spores are not germinated prior to their death. The mechanism of spore death appears to be CO_2-facilited disruption of the exosporium, causing uptake of the H_2O_2 and oxidation of the spore membranes. Both titanium-based orthopaedic implant materials and expanded Teflon ™ vascular implant materials remain biocompatible after CO_2 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 CO_2 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 CO_2; therefore, medical devices fabricated from such plastics may not be compatible with CO_2 sterilization. Visual observation shows that CO_2 + H_2O_2 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 CO_2; thus, in future work more attention must be given to facilitating contact and mass transfer in such geometries.

Conclusions Further results appear in the following articles:

Effects of sterilization on implant mechanical property and biocompatibility. An, Y.H.; Drews, M.D.; LaBerge, M.; and Matthews, M.A. *International Journal of Artificial Organ*, 2005, 28, 1126-1137

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. *Journal of Separation Science*, 2005, 28, 1642-1647

Biocompatibility of supercritical CO2-treated titanium implants in a rat model. Hill, C. M., Kang, Q. K.; Wahl, C.; Jimenez, A.; Laberge, M.; Drews, M.; Matthews, M. A.; and An, Y. H. International Journal of Artificial Organ, 2006, 29, 430-3.

Sterilization using high-pressure carbon dioxide. Zhang, J.; Davis, T. A.; Matthews, M. A.; Drews, M. J.; LaBerge, M.; and An, Y. H. *Journal of Supercritical Fluids*, 2006, in press.

STATUS OF RESEARCH AND PARTNERSHIP

The current Bioengineering Research Partnership is in a no-cost extension to May 2006. Seven refereed papers have been published or accepted, and one is to be submitted, and one more is in preparation. In addition, eleven presentations at professional meetings have been made. Industrial interest remains strong; two invention disclosures have been submitted, and a small business has been formed at the University of South Carolina.

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 (NIBIB)

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. Known as the MIDAS project (Metabolic Imaging Data Analysis System), the developed software includes MRSI processing and metabolite image reconstruction, tissue segmentation, spatial transformation and brain region mapping, statistical analysis, and clinical presentation. Results from MRSI and MRI studies are 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 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.

STATUS OF RESEARCH AND PARTNERSHIP

In this fourth year all of the major program modules have been developed and integrated to form a single set of processing tools that can be run in a pipelined processing environment. All MRSI and MRI processing can be carried out in a fully automated manner. Organization of individual subject data and project-level information is based around a XML-based data-management system. This is integrated into programs written in different programming languages by making use of a common set of library functions written in Java.

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 and 3.0 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.

The developed software has now been released to a limited number of sites. In addition, to expand data collection a volumetric MRSI acquisition sequence is being made available for Siemens MR instruments. Following completion of a data sharing agreement, the acquired data will also be made available.

The aims of this project and the partnership groups remain unchanged.

ISSUES

The continued evolution of MR methods and instrumentation has required frequent reassessment of the required functionality. For example, the use of phased-array acquisition methods required additional support for this data acquisition method and additional data processing for the MRI processing methods. In addition, an alternative spectral intensity normalization method is under development and modifications.

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PROJECT TITLE: Leukocyte Trafficking: From Flowing Blood to Tissue

PARTNERS' NAMES AND AFFILIATION:

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

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

We have used microarray analysis to investigate changes in neutrophil and endothelial cell gene expression due to transmigration. Briefly, endothelial cells were grown on PTFE membranes containing 3 um pores. Endothelial cells were stimulated with IL1b for 4 hours followed by application of freshly isolated neutrophils. Transmigrated neutrophils were collected after 1 hour of neutrophil transmigration. mRNA was isolated from both neutrophils and endothelial cells. EC gene expression after ILb treatment and neutrophil transmigration was compared to that of EC gene expression after IL1b treatment alone.

The gene expression of transmigrated neutrophils was compared to that of neutrophils which did not migrate across the EC layer and to freshly isolated neutrophils. Microarray analysis of mRNA from endothelial cells indicated that IL1b treatment causes differential expression of 1.5 fold or more in over 2500 genes. Neutrophil transmigration after IL1b treatment had a much less dramatic effect on endothelial cells, causing differential expression of 1.5 fold or more in only 23 genes. Microarray analysis of mRNA from transmigrated neutrophils revealed differential expression of 511 genes when compared to mRNA from freshly isolated neutrophils. Comparison of mRNA from neutrophils which migrated across the endothelium to mRNA from neutrophils which failed to migrate revealed differential gene expression of 144 genes. We are in the process of confirming these differentially expressed genes via qRTPCR. Genes which may have significant functional contributions to leukocyte migration will be investigated further.

For studies of neutrophil rolling and adhesion under flow conditions, we have wanted to have surfaces that can present several ligands in defined patterns on a non-adhesive background. In our prior studies (see Gonzalez et al.,2004), we have demonstrated that PEG hydrogels are intrinsically non-adhesive for neutrophils yet can be rendered adhesive when modified with appropriate peptides. Thus, we are utilizing these types of materials in flow studies. We have developed technologies that allow patterning of multiple ligands (such as RGDS and sialyl Lewis X) in defined geometries with micron scale resolution using photolithography and laser scanning lithography. Photolithographic approaches are most straight forward but limit the number of ligands that can effectively be patterned. Laser scanning lithography has allowed us to overcome this limitation. A three dimensional 2-photon laser scanning lithography technology has also been developed (publication in press), which will have use in migration studies described below. We have demonstrated that these hydrogels can be utilized in parallel plate flow chambers to study defined leukocyte interactions (publication in revision).

The PEG hydrogels are also attractive because the same materials can be rendered proteolytically degradable, potentially allowing one to study transmigration through an ECM analog following rolling and firm adhesion. Endothelial cells can also be seeded on the surface of an appropriately modified PEG hydrogel. To aid in the study of proteolysis during transmigration, we have developed hydrogel materials that become fluorescent upon cleavage by targeted proteolytic enzymes. The fluorescent products remain bound to the material, allowing one to assess the spatial localization of cellular proteolytic activity. Within these same materials, we can also covalently immobilize gradients of chemotactic agents or create three-dimensional patterns that present bioactive moieties in defined context.

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 May 15-16, 2006 in Houston. Our next one will be in April of 2007. 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 (NIBIB)

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. 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. 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

This period was a no cost-extension of a 3-year BRP program. Effort during this period was directed predominantly at in-vitro application of nanofiber based electrode architectures for electrophysiological and electroanalytical probing of neuronal tissue. Several embodiments of individually addressed nanofiber electrode arrays were evaluated with neuronally differentiated cultures of rat pheochromocytoma (PC-12) and hippocampal slices from embryonic rat. Dual modality measurements of both membrane depolarization and extracellular dopamine release were obtained at discrete nanofiber electrodes using both differentiated and undifferentiated cultures of

PC-12 in response to stimulus applied either via electrical input into the nanofiber electrode or by application of secretagogue (1 mM nicotine). This study, currently in press in J. Phys. Chem. B, provides basis of use of penetrant nanofiber electrode arrays for multimodal (chemical and electrical) neuronal interfacing to intact tissue. Use of such penetrant electrodes may minimize or eliminate some of the problems associated with the interface between conventional neural prosthesis and excitable tissue (i.e. necrosis and gliosis of the interfacial region), and may provide higher spatial resolution due to the ability to generate electrodes with nanoscale diameters. Further development of these techniques for interfacing to intact hippocampal slices are proposed in a competing R01 in collaboration with Dr. Nance Ericson, ORNL, and Dr. Barclay Morrison of Columbia University.

In addition to cell studies, we have developed a new approach at nanofiber electrode array fabrication that is more suited to clinical application of these arrays. Previously, nanofiber-based devices developed in this effort have been limited to planar geometries using substrates of silicon, fused silica, and quartz. These planar devices have proven effective for cellular interfacing in-vitro, as platforms for both electrophysiological and electroanalytical probing of neuronal cultures as well as effective vectors for DNA delivery to mammalian and plant tissue culture. Planarity of these substrates, however, does limit the ultimate clinical application of these devices. As such, we directed attention at developing techniques to transfer electrically addressable nanofiber based devices into flexible polymer membranes such that the nanofiber electrode arrays and implants incorporating these arrays might be interfaced more readily with tissue. The resulting flexible polymer nanofiber arrays, featuring individually addressable high aspect ratio nanofiber electrodes, are documented in Fletcher et al. Adv. Mater. 2006, 18, 1689–1694.

The underlying technology developed during this BRP has spun out into several new areas of research. In addition to our collaboration with Dr. Barclay Morrison, Columbia, we have initiated collaboration with Drs. Eldon Geisert and Elizabeth Fitzpatrick of the Hamilton Eye Institute, UTHSC. They have initiated biocompatibility assays of nanofiber arrays (endotoxin, BrDU, MTT) towards application of these arrays for ocular gene delivery. Dr. Ram Mahato, also of UTHSC, is currently evaluating VACNF arrays as a vector for gene delivery to pancreatic islet cells. Dr. David Price, U of Iowa, is implementing penetrant nanofiber scaffolds to augment his program studying transcriptional elongation factors. We are also combining the gene delivery and electrochemical aspects of these probes to attempt to impart electrochemical influence over transcription of tethered template on nuclear penetrant nanofibers. This is the subject of a 3 year R01 that resulted from the efforts of this initial BRP. Additionally, using techniques developed through the BRP mechanism, nanofiber electrode material is being implemented by a small business, Nanotek LLC, for initial stages of processing of cyclotron irradiated 0-18 water in a microfluidically-based positron emission tomography (PET) radiotracer synthesis platform.

ISSUES

None.

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PROJECT TITLE: Quantitative Bioengineering Analysis of Muscle Mechanics and Metabolism

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

ABSTRACT

Using the methods of engineering analysis, we are developing a computational platform that incorporates current knowledge of molecular structure, biochemical energetics, and contraction kinetics to describe muscle contraction. Our goal is to develop a comprehensive model that can be used to (1) generate new mechanistic hypotheses concerning the functions of contractile proteins myosin and actin, and (2) quantitatively evaluate the roles of accessory and regulatory proteins in muscle contraction. Once developed, the model will be a powerful analytical and predictive tool in studies of muscle contraction. Presently, no models of contraction account for complications due to both (1) extensibility of the actin and myosin filaments, and (2) Ca^{2+} regulation of contraction. Filament extensibility results in non-uniform load transfer along the thick and thin filaments, which introduces variability in the stress and strain of the myosin heads during their interactions with actin. These effects must be taken into account to understand how cross-bridge forces affect chemical transitions in the actomyosin ATPase cycle and *vice versa*. Further, quantitative understanding of Ca^{2+} regulation will allow for more accurate (1) predictions of the macroscopic mechanical and energetic consequences of specific regulatory events, and (2) explanations of macroscopic events in terms of underlying molecular processes. We will address these problems via a multidisciplinary approach that spans engineering science, computational science, and biophysics and rests entirely upon first principles. Our team will develop a model of contraction that integrates a critical missing element, filament extensibility, with recent advances in understanding the (1) biochemical states of myosin, (2) state transition rates of the actomyosin ATP hydrolysis cycle, (3) function of myosin molecular motors in the thick and thin filament lattice (sarcomere), and (4) Ca^{2+} regulation of myosin binding. Initially, the model will combine probabilistic or stochastic actomyosin binding kinetics with finite element analysis (either continuous or spatially discrete model consistent with the periodicities of the thick and thin filaments). The model will then be refined to explain smooth muscle contraction, including the energetically efficient latch state and the actions of proteins involved in the regulation of contraction. The computational model developed here will invoke unifying principles that apply to the actomyosin interaction cycle regardless of muscle type but will have sufficient flexibility to account for contraction kinetics and regulation of contraction in different muscle types. Quantitative modeling of contraction is ultimately essential for understanding the molecular basis for a wide range of syndromes and diseases, such as airway narrowing in asthma, and weakness of both heart and skeletal muscles in heart failure.

STATUS OF RESEARCH AND PARTNERSHIP

During year three our research has progressed as planned. We already have published seven papers and we have four more in review. We have also presented many abstracts at national and international conferences in 2005 and 2006.

Specific Aim 1 is on schedule: (1) we continue development of the computational platform that combines stochastic three-dimensional discrete actomyosin binding with finite element analysis. The developed mechanokinetic models for the muscle half-sarcomere which includes: (i) extensible filaments, (ii) allow all feasible head-site of actin-myosin interactions in the 3-D filament lattice, (iii) discrete head positions on myofilaments, (iv) heads bind to target zones on Factin, and we implemented and updated eight state actomyosin cycle. This new model brought up many important issues that were not observed in the other models. In our comprehensive analysis we used a combination of an approximate mean-field approach in terms of independent state-occupation probabilities for myosin heads (Dr. Smith, Monash University, Australia) and stochastic (Monte Carlo) simulations (the Harvard group). Some of our results contradict several currently accepted notions. Accordingly we are developing a set of new experiments which could reconcile these contradictions. (2) Dr. Geeves (University of Kent, UK) continues his investigation of the correlation of biochemistry and mechanical data on the cross bridge cycle.

Specific Aim 2 is also on schedule: (1) we have developed the first stochastic model of the thin filament regulation in solution. This model includes "classical" rigid tropomyosin model and a novel flexible chain model. The latter is developed from the first principles and the model predictions of cooperativity showed much better quantitative agreement with the experiments. We are now working on establishing dependence of the model kinetics parameters on calcium concentration. We also developed tolls for evaluating dependences between the parameters. However, we found that some model parameters cannot be uniquely resolved. Thus, we are now designing a set of experiments that could, potentially, uniquely resolve all the model parameters. In parallel, we are working on implementation of a calcium (transient) regulation in a 3D sarcomeric lattice; and (2) Drs. Moss and Fitzsimons are working on altered thin filament cooperativity and cross-bridge kinetics due to expression of truncated cardiac troponin.

Specific Aim 3 is ahead of schedule: we are now developing a model of airway narrowing which includes dynamics of breathing in whole lungs. The computer simulations include contraction and regulation of airway smooth muscle, current geometry of an airway during excess airway narrowing, and instantaneous pressure drop along airway tree during breathing.

The BRP partners met for a two-day meeting (Madison, WI, August 2005) and one and half-day meeting (Salt Lake City, February 2006). These meetings have been intense, mutually beneficial and highly productive. At the former meeting we discussed progress in previous year and we defined the milestones to be achieved in the Year 3. At the latter meeting we reviewed the progress since Madison, WI, meeting and discussed critical issues related to actomyosin cycle, three-dimensional discrete myosin binding, stochastic thin filament regulation, and design of critical experiments which will provide important model parameters and data for model testing. We planned coordinated work at each site for Year 4, and also we prepared outlines for several papers that we plan to submit for publication this year. Four of these papers are in review and other four are in preparation. We continue to develop new software, hardware, and experimental protocols.

ISSUES

None.

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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 Communications Disorders (NIHDCD)

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, annual 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 our NIH/NIDCD Program Manager.

The primary advances made during FY 2005 are the successful demonstration of an optical sensing scheme that provides an electronic signal that is proportional to the displacement of the directional microphone diaphragm as it responds to sound. A miniature prototype package assembly has been demonstrated resulting in an initial prototype of an integrated microphone with a miniature version of the optical sensor electronics and a simple, nondirectional microphone diaphragm. The sensor electronics utilize a semiconductor laser and photodetectors that have been integrated and optimized for low power consumption and will be part of the final electronics design chip.

Several microphone designs were successfully designed, optimized, fabricated and tested. These designs included microphones that utilize conventional capacitive sensing as well as microphones that incorporate optical gratings to allow an optical detection of the diaphragm displacement. Acoustic testing of these parts shows a very satisfactory performance.

ISSUES

Some of the challenges encountered this year have been the re-characterization of a polysilicon furnace, tool difficulties and equipment down time at our nano-fabrication facility.

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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, Musculoskeletal, and Skin Diseases (NIAMS)

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.

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 times are relatively slow (hours), this issue will be addressed in the coming sets of experiments.

Since DEI and MIR are transmission radiographic techniques they depict 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. Our work this past year focused on a comparison of results obtained between DEI and Magnetic Resonance Imaging for intact human cadaveric knee joints. These 32 joints had been first imaged through MR and were then formalin preserved (a method that does not effect the results of DEI) and then imaged with DEI at Brookhaven National Laboratory. We found very good correlation between lesions observed with the two different technologies. 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 oblique and medial/lateral plane as well. This, however, does not allow visualization of most of the cartilage surfaces that are superimposed by bone at all views. This is the disadvantage of comparing DEI, a planar imaging technology to MRI (a tomographic/three dimensional modality). Thus the need to develop DEI in the CT mode, which is the goal of the impending resubmission of the competitive grant renewal. We know that we can attain a resolution with DEI (10 microns) that is higher than that obtainable through MRI, and thus, the imaging of the earliest morphological changes in the cartilage surface will be detected with DEI and not with MRI.

This past year we also completed the component of the study in which investigators at Massachusetts Institute of Technology (MIT) in which the following were found:

- The structural changes in human and bovine cartilages after 50% and, more so, after 80% compressive injury led to a loss of tissue thickness that was associated with significant changes in parameters of density, texture and homogeneity in DEI images as well as with an increased articular cartilage damage score.
- These findings were consistent with irreversible tissue compaction and structural damage to cartilage and its collagen network.
- These changes were more profound for immature bovine compared to the adult human tissue and, in turn, were more dramatic for human distal femur compared to talar cartilage (Figs. 1,2).
- These findings were also consistent with recently reported results [7] showing that injurious compression led to different responses in degenerated human knee versus ankle cartilages: in macroscopically intact cartilages of degenerative articular surfaces, increased dynamic stiffness was observed in tissues in which the most compaction had occurred following injurious compression.

This work is currently in preparation for publication.

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. We have advanced the DEI methodologies to the Multiple Imaging Radiography technology and have thus made the computed tomographic mode possible. Our collaboration with MIT has also brought us into new territories by demonstrating that DEI is capable of allowing visualization of compressive cartilage injury, albeit in the ex-vivo situation. It is necessary for further development of Multiple Image Radiography, in the computed tomographic mode, to be optimized for the full potential of these technologies to be realized for the imaging of early cartilage lesions.

ISSUES

The one major issue over the past two years has been getting one of the smaller subcontracts to follow through with the obligations to the grant. We have had to replace the PI on this subcontract so that the required data could be obtained for grant fulfillment and for resubmission of the competitive renewal this summer.

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PROJECT TITLE: MR Image Analysis in MS: Identification of a Surrogate

PARTNERS' NAME AND AFFILIATIONS:
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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

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

We have completed the development and validation (using data acquired on different field strengths from multiple centers) a unified technique for segmenting lesions, gray-matter, white-matter, and CSF. The segmentation technique is based on conventional MRI sequences that are routinely used in clinical practice in MS and is machine independent. We have completed the development and implementation of techniques for automatic detection and quantification of black holes and Gd enhancements in MS. Currently we are testing the performance of these two methods using the multi-center data.

A. Partial Volume Averaging: Accurate classification of gray matter (GM) and white matter (WM) is an important step in the estimation of atrophy of these two tissues in MS. However, even when the relatively small slice thickness of 3 mm, the partial volume averaging between different tissues often results in false classifications and affects the estimated tissue volumes. In order to minimize this problem,

a technique based on partial volume averaging along with the bias field correction is developed to improve the classification of GM and WM in MS. The partial volume averaging correction significantly improved the quality of segmentation.

Abnormal White Matter: Part of the white matter MS appears normal on conventional MRI is known to be pathological. This so called “normal appearing white matter (NAWM)”, which is particularly prominent around the MS plaques, could be related to the clinical disability. Quantitative evaluation of NAWM could be particularly important in the identification of a surrogate in MS. We have developed a technique based on the combination of diffusion tensor imaging (DTI) and multiparametric segmentation of MRI for quantification of the NAWM in MS brains.

Detection of Gray Matter lesions in MS: Detection of purely intracortical lesions in multiple sclerosis (MS) with conventional MR imaging remains a challenge. While double inversion recovery (DIR) techniques, based on fast spin echo sequence, have been shown to improve the detection sensitivity of intracortical lesions, this sequence is prone to image artifacts and poor lesion border delineation. In these studies we have shown that intracortical lesions can be identified with greater confidence by combining DIR with phase sensitive inversion recovery (PSIR) images.

Combined PSIR+DIR showed a 337% improvement in total number of lesions detected (306) compared with FLAIR (70). Intracortical lesion detection (124) was improved by 417% compared with FLAIR (24). Detection of mixed grey/white matter and juxtacortical lesions (144 and 39) was improved by 396% and 130 % respectively relative to FLAIR (29 and 17). PSIR consistently showed a much clear delineation of lesion borders and more confident distinction between purely intracortical, mixed grey/white matter and juxtacortical lesions.

Reliability in visualization of intracortical lesions in MS can be greatly improved by combined use of PSIR and DIR techniques. Accurate detection and classification of these lesions is important in understanding their role in disease progression and impact on the clinical manifestations of the disease.

Software: As proposed, we have completed the development and testing of all the software under the Windows environment. The software is designed to be modular and user-friendly.

ISSUES

The key personnel from Brain Insights, a subcontractor on the parent grant, have left the company and the subcontractor indicated the inability to continue working on this project. Therefore, Brain Insights is no longer a subcontractor on this project. This work will be now will be carried out by the PI's research group. The PI's group has enough expertise in this area.

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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 (NCI)

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 the following areas: (1) molecular profiling of cancer cells and tissue specimens; (2) clinical outcome database; (3) cancer biology; (4) molecular beacon development; (5) spectral imaging instrumentation; and (6) bioinformatics and data analysis. In particular, we continue to make significant progress in using semiconductor quantum dots (QDs) for multiplexed molecular profiling of prostate cancer cells and tissues obtained from animal models and clinical tissue specimens. We have focused our efforts on epithelial to mesenchymal transition (EMT) because of the importance of this molecular process that could lead to increased cancer cell migration, invasion, and metastasis. Taking advantage of our success in the development of the first human prostate cancer EMT model (Xu et al., Prostate, 2006, in press), we have completed a series of analyses using antibody-QD conjugates for the detection of biomarkers on the cell surface in a multiplexed manner. We have now completed a study where we have demonstrated that, in addition to vimentin, E-cadherin, and N-caderin, IL-13 receptor 2 and RANKL are two novel EMT markers that are associated with the ARCaP EMT model. We have validated these markers by the use of multiplexed QD approach in tissue specimens harvested from mice with metastatic prostate cancer and also from clinical specimens.

In tumor metastastasis and biology studies, we have examined intracellular signaling pathways that may control androgen independent progression of prostate cancer cells, and we embarked on elucidating the molecular mechanism underlying osteomimicry of prostate cancer cells. We discovered that a previously unknown cell signaling pathway linking between a soluble factor, 2-microglobulin, which binds to an unknown cell surface receptor prior to propagating cell signaling through protein kinase A, cyclic AMP responsive element binding protein (CREB), and vascular endothelial growth factor (VEGF) axis as important control for the growth and survival of human prostate cancer in the primary and at metastatic sites (submitted to *Cancer Research*). The documentation of this signaling pathway opened up the opportunity for therapeutic targeting and also for the prognosis and diagnosis of lethal prostate cancer bone metastasis. Following the documentation of this signaling pathway, important for prostate cancer survival, we demonstrated that disruption of this signaling pathway resulted in prostate cancer death. In this coming funding period, we will begin to explore the possibility of detecting the signaling cascade using multiplexed antibody QD conjugates.

In clinical patient outcome studies, we have provided test prostatectomy tissue for the perfection of multiplexed marker analysis. This involves identifying blocks in the pathology department that have both cancer and benign tissue in the same section, cutting a series of 30-40 consecutive slides, and staining every 10^{th} slice with H&E for accurate identification of areas of cancer. These series of tissue slides are then used for multiplexed marker analysis. The markers chosen for these first experiments are two that have substantial original research from my laboratory (DEFB-1 and NOX-1) and also PSA. Following staining with nanoparticles, this project also provides pathological expertise in interpretation of quality of staining, cell type specific staining etc. We are pleased to report that 3-plex staining has been accomplished and refined to standard protocols.

The work of this partnership has led to a number of joint publications in cancer nanotechnology and biomolecular engineering.

ISSUES

None.

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PROJECT TITLE: Raman Flow Cytometry for Diagnostics and Drug Discovery

PARTNERS' NAMES AND AFFILIATIONS:

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

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 microbial 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

Several key milestones were achieved in Year 2 of the Partnership in the areas of instrumentation, reagent development, and biological applications.

Instrumentation**.** The first key instrumentation goal was to modify a commercial large particle sorter to enable detection of discrete Raman vibrations. After evaluation of various configurations of excitation and emission optics on an optical table, an optimal design was implemented on the commercial sorter. This configuration features delivery of 633 nm excitation from a HeNe laser via an optical fiber to the excitation optics of the sorter. Raman scatter is collected at 90 degrees to the excitation beam via high numerical aperture aspheric lenses, coupled into an optical fiber, and delivered to a high throughput imaging spectrograph. The dispersed light is imaged onto an array of optical fibers and the output from individual fibers is detected using a photomultiplier tube (PMT). The PMT signals are processed through a pre-amp and sent to the data acquisition electronics of the commercial instrument. Instrument alignment and sensitivity are monitored using fluorescent microspheres developed for this purpose. Spectral response is calibrated using a calibration lamp. Using this system we were able to detect discrete Raman

vibrations from polymer beads bearing SERS-nanoparticles. This represents the first ever detection of discrete Raman vibrations by flow cytometry. Current efforts are aimed at increasing signal to noise through higher power excitation sources, lower noise detectors and electronics, longer integration times, and brighter nanoparticles.

The second key instrumentation goal is to develop a new system that is capable of analyzing full Raman spectra rather than discrete vibrations. This goal will be pursued full time starting in Year 2, but preliminary work is underway. Using a standard flow cell configuration, excitation and collection optics similar to that described above, and a loaned electron multiplied CCD camera, we demonstrated the ability to collect spectra from SERS nanoparticles (estimate <2000 particles) in a hydrodynamically focused sample stream with integration times on the msec times scales. This performance is quite compatible with many biological applications, but we expect we could improve speed and sensitivity by nearly two orders of magnitude with straightforward optimization. We are currently evaluating potential cameras and data acquisition approaches for full spectral analysis on flowin anticipation of increased effort in Year 3.

Micro- and Nanoparticles. With the instrument development aspects well underway, the development of micro- and nanoparticle reagents becomes critical. In the past year we made significant progress in optimizing approaches to prepare colloidal gold aggregates with adsorbed Raman tags for use as SERS tags and to stabilize these with thin glass coating. We have established methods to functionalize the glass coating with biotin, amino, and carboxyl groups for attachment to biomolecules. We are currently working on increasing the intensity of the SERS signal and increasing the number of tags to give particles with distinct Raman spectra. We are also exploring ways to enhance Raman scattering from polymer resins used for combinatorial chemistry, with a couple of promising approaches that are being pursued.

Biological Applications. The biological applications of the Partnership involve the development and application of ligands and substrates for use in diagnostics and therapeutics for microbial pathogens. This work is being led by Drs Nolan (LJBI) and Graves (LANL). In the past year we have completed the screening of a phage display library for peptides that bind to cholera toxin, using a new high throughput multiplexed screening approach (manuscript in preparation). The peptides identified by this approach have been synthesized on solid supports and the development of protocols for screening of synthetic peptides on resins by flow cytometry is underway. We have also developed a phage library and methods for the identification of peptides that are cleaved by proteases. We are using this library to screen for substrates of Bacillus anthracis lethal factor, which will then be optimized by combinatorial chemistry as for binding ligands.

ISSUES

The Partnership is functioning very well. Members meet twice a year in La Jolla to formally review progress, set milestones for the coming period, and explore new opportunities. Periodic teleconferences focus on specific aspects of the project, and email serves as the most frequent mode of communication. No major problems have been encountered thus far.

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PROJECT TITLE: Adaptive Optics Instrumentation for Advanced Ophthalmic Imaging

PARTNERS' NAMES AND AFFILIATIONS:

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GRANTING NIH INSTITUTION/CENTER: National Eye Institute (NEI)

ABSTRACT

The goal of this BRP is to combine adaptive optics and confocal imaging to create instruments for noninvasive imaging of the retina with unprecedented spatial resolution. The first four instruments are:

1. The Rochester AOSLO, capable of fluorescence imaging, that can image eyes, such as those of rat, dog, and human, with different pupil sizes and focal lengths. This instrument is now in use not only for projects at Rochester but also in collaboration with John Flannery's group at UC, Berkeley.
2. The UC, Berkeley AOSLO that can track the pupil and thereby relax the requirement for head stability in patients. This instrument incorporates eye movement dewarping methods developed through a subcontract with mathematicians at the University of Montana.
3. The Lawrence Livermore AOSLO, developed for clinical researchers at the Doheny Eye Institute. This instrument uses two cascaded deformable mirrors to provide an increased range of correction for ocular aberrations. This instrument was delivered to the Doheny Eye Institute in May 2006.
4. The Indiana AOSLO that will incorporate heterodyne detection, polarimetry, and retinal tracking to increase system performance. Retinal tracking has been implemented through a subcontract with Physical Sciences, Inc., and improvements in the technology are ongoing.

STATUS OF RESEARCH AND PARTNERSHIP

Functional instruments capable of collecting retinal images at high magnification have now been demonstrated at all 4 sites originally contracted to build instruments. 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. Inter-institutional collaboration is facilitated by video/teleconferencing as well as face-to-face meetings. For example, BRP sites participated in a one-day retreat at the OSA meeting in Tucson in Oct. 2005.

Instrumentation Highlights:

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 has capitalized on the efforts of the Center for Adaptive Optics (CfAO), an NSF-funded Science and Technology Center, which has worked with several companies to develop new deformable mirrors for vision science applications. Based on the outcome of these developments, the BRP selected Boston Micromachines mirrors for all four of its instruments. Boston Micromachines mirrors with 3.5 microns of stroke have now been implemented in all four systems. In addition, LLNL has incorporated a second

compact mirror technology provided by a company, AOptix, with 15 microns of stroke, making it easier to image clinical patients.

Mitigating against eye movements. Roorda's group at UC, Berkeley, working with the University of Montana, and Burns' group at Indiana University, working with Physical Sciences, Inc., have made important progress toward tracking the retina and removing the image artifacts caused by eye movements. Roorda's group has demonstrated that adaptive optics can help create the most accurate measurement of eye movements ever made, and the Montana group has recently succeeded in real-time dewarping of AOSLO retinal images. The Indiana group has demonstrated hardware tracking in real time to within 15 microns, and automated offline alignment to 1 micron, and Physical Sciences, Inc. has demonstrated the capability for automated montaging of the retina using their system. These capabilities may have important applications well beyond the immediate goals of the BRP, possibly allowing the stabilization of therapeutic lasers in the eye, enabling light delivery with far greater accuracy than currently achieved.

Scientific Highlights:

Imaging RPE autofluorescence. The Rochester group has imaged individual RPE cells in the living primate and human retina using autofluorescence. 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 developed an impressive arsenal of fluorescent markers to stain photoreceptors, ganglion cells, and Muller cells. Moreover, his group has developed fluorescent reporters that may ultimately allow the BRP to image neural activity in living primate ganglion cells.

Cone loss and visual acuity in inherited retinal disease. Austin Roorda's group at UC, Berkeley has succeeded in quantifying cone loss in retinal degenerations and has shown that massive losses can occur before patients show losses in visual acuity.

Significance:

The BRP has now developed four instruments that allow noninvasive microscopic imaging of the living mammalian retina. Though these instruments all share adaptive optics and confocal imaging capabilities, each is optimized to have different capabilities, such as fluorescence or polarization imaging. The key advantage of these instruments is that they allow us to examine retinal disease at the cellular level in living eyes, something that has before now generally required microscopic examination of post mortem tissue. The technology promises to allow new studies of normal retina, continuous monitoring of disease progression in abnormal retina, and evaluation of the efficacy of retinal therapies. Moreover, our improving capabilities in eye tracking in combination with high resolution retinal imaging may allow improvements in therapeutic light delivery.

ISSUES

Two of the principal investigators in the BRP completed moves of their laboratories last year. Austin Roorda moved from U. Houston to U. Berkeley effective Jan. 1, 2005 and Steve Burns moved from the Schepens Eye Research Institute to Indiana University at the end of the 2nd quarter, 2005. Both laboratories are up and running productively following these moves.

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PROJECT TITLE: New Magnetically Suspended LEV-VAD

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

ABSTRACT

The value of magnetically suspending the impellors in small rotary blood pumps is well recognized and has been utilized in at least three different clinically used radial (centrifugal) blood pumps. The flow paths are complex and no one has been successful in eliminating the often stagnant area behind the rotating disc impeller. The flow path or paths in axial flow pumps have proven superior in several ways including the elimination of all complex recirculation zones and obstructed flow paths.

The limited cardiac donor supply emphasizes the critical need for developing a reliable functional LVAD to be used as either a bridge to myocardial recovery (BTR) or more importantly, destination therapy. This proposed multi-year effort involves the final development and experimental testing of a compact, axial flow, magnetically LEVitated impeller, left Ventricular Assist Device (LEV-VAD) for clinical use. In contrast to currently available rotary VADs, this team has designed an axial flow LEV-VAD with an unobstructed blood flow path, thus eliminating retrograde flow, stagnant areas, and without mechanical support bearings.

STATUS OF RESEARCH AND PARTNERSHIP

Our new prototype axial flow LEV-VAD has a diameter of 40 mm and a length of 120 mm, which makes it very small. The design goal is for a 20 year life expectancy that we believe will be difficult to attain by the larger, mechanical pulsatile VADs with flexing diaphragms and valves, or the rotary pumps with mechanical bearings. To achieve our goal of developing a VAD with a 20-year lifespan, our two main objectives will be to eliminate mechanical failures by utilizing long lasting magnetic non contacting/wearing bearings with a minimized power consumption and wire count; and second, to eliminate thrombosis with magnetic suspension of the impeller with a single pass, non-obstructed high flow through the pump.

The primary goal and objective of this project is to develop and validate the LEV-VAD with sufficient documentation to submit an application to the FDA for Investigational Device Exemption proof of performance for clinical trials. After preliminary results the device will be implanted into a series of healthy calves for six hours to assure the anatomic fit and assess function in the instrumented calves. A major effort will be to develop a responsive physiologic controller initially by monitoring axial position as measured by Hall effect sensors. Preliminary studies have demonstrated that the readily available axial

position, motor speed and motor power can be used for a very sensitive physiological controller. We have a high level of confidence that we will know the boundaries for the application and limitations of the LEV-VAD as a destination therapy device at the completion of these proposed studies.

Specifically, the following design objectives will be demonstrated: 1) The innovative, implantable axial flow LEV-VAD design [Figure 1] will provide a compact pump suitable in size with the capacity to meet the needs for the ventricular support of adult cardiac failure patients. 2) The LEV-VAD system will have an expected lifetime of nearly 20 years, making it suitable for destination therapy. 3) As a marked improvement over currently available rotary VADs, the blood path will be streamlined by creating an unobstructed one-pass design without recirculation paths focused to minimize the incidence of hemolysis and stasis leading to thrombosis. 4) The one-pass blood path will be achieved by using an innovative combination of high strength permanent magnets and one actively control magnetic bearings. This advanced suspension system has several advantages, including insuring impeller levitation at start up and during operation, no mechanical wear, and a significant reduction in wire count, controller and battery size, and power consumption by using primarily permanent magnets. The permanent magnet thrust bearings are able to maintain the impellers in an optimally centered position without projecting into the blood path even when subjected to variable axial pressure loads. An efficient, brushless, 3-phase DC motor with an innovative magnetic path design that ensure unidirectional start-up and operation will be incorporated into the LEV-VAD support system. Additionally, an active physiological sensor/controller system for the pump will be explored to ensure: a) Adequate cardiac output to ensure tissue perfusion and support of native cardiac function with automatic adjustment for physiologic changes due to stress, activity, and left ventricular recovery. b) Avoidance of left ventricular collapse due to mechanical suction from the pump. c) Sufficient pump speed control to avoid retrograde pump flow due to aorta-left-ventricular-pressure differential.

The initial design of the prototype pump has been frozen and the majority of components of the pump system that we refer to as R0 have been fabricated. Extensive numerical simulations have demonstrated that both the magnetic (Finite Element Analysis) and fluid (Computational Fluid Mechanics) systems will meet the design specifications. We are currently performing detailed empirical validation all components of the sensor system, magnetic suspension, and fluid handling elements before completing final assembly of the prototype pumps.

In parallel to the above effort, we have also been working on a second design iteration that in its fluidic concept follows the same component structure layout (Inducer - Impeller - Diffuser) in that Form Follows Function. However, this new design, which we refer to as the R2, incorporates several new and innovative magnetic component structures that simplify and streamline the fluidic component design, while simultaneously improving and refining the magnetic performance.

After extensive bench and mock circulation loop testing of both versions of this LEV-VAD (R0 & R2), we anticipate the first animal implants will occur in the fall of this year.

ISSUES
None.

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PROJECT TITLE: Development of Networked Implantable Neuroprostheses.

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GRANTING NIH INSTITUTES/CENTERS: National Institute of Neurological Disorders and Stroke (NINDS), National Institute of Biomedical Imaging and Bioengineering (NIBIB)

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 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 in meeting the technical needs of a broad range of neuroprosthetic applications and can be scaled for each new application through the selection and interconnection of the appropriate functional modules.

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, eliminates external components, and can be easily implemented using current surgical techniques.

STATUS OF RESEARCH AND PARTNERSHIP

Our project was initially funded through NINDS within which we essentially conducted a proof-of-concept study during the first two years. We are currently in the second year of an additional five years of

funding through NIBIB. This second round of funding will 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.

We have identified and described four FES operating environments that must be addressed and accommodated by the NNPS technical design. They include the *surgical*, *clinical*, *user*, and *research* environments: the *surgical* installation environment limits access and communication methods; the *clinical* environment requires transparent, real-time, programming access to all user implanted components; the *user* environment must provide transparent, robust, non-tethered operation; the *research* environment requires high-bandwidth communication and processing capabilities. The challenge is to satisfy the technical and operating needs of each of these environments as fully as possible.

To date, we have focused on addressing the critical concepts and designs that required new knowledge and/or techniques (including forming the necessary partnerships). We have established overall design topology and system requirements, identified needed system components (hardware and software), developed (and continue to develop) network communication protocols, addressed powering issues, and initiated hardware designs and prototypes.

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 having power-transfer/data-communication, multi-drop, network cables, providing a scaleable network infrastructure for connecting multiple actuator-type and sensor-type modules. This infrastructure will support totally implanted closed-loop systems – an important goal of the distributed system concept.

Testing of the feasibility of Li-ion rechargeable cells as the central power source for the NNPS is essentially completed. We have selected a commercially available, implantable grade, Li-ion cell (Quallion, Inc) and are configuring our hardware designs for its use. Ongoing cell testing is focused on repeating the results to date and fine-tuning the performance and operational criteria of the selected Li-ion cell. The major goal of this testing is to determine suitable limits and methods for charging and discharging to maximize the cycle-life performance of the cell.

We continue to pursue the design of the network cable – a critical element of the NNPS. Significant mechanical testing and evaluation of silver-cored leadwire and cable structures and their configuration is ongoing. Results to date are encouraging. We believe that we will be able to custom fabricate a leadwire structure and design a multiconductor cable to meet our needs – mechanically and electrically. We have also initiated a short study to investigate the in vitro corrosion behavior of silver-cored leadwires.

We have recently begun hardware fabrication of several system components, moving these system elements from the design and concept stage to the design verification stage.

We continue to have strong relationships with our existing consortia, providing the necessary industrial and academic partnerships in order to complete the design and fabrication of the NNPS. We have recently partnered with SBC, Inc. The principal Investigator from SBC, Sonny Behan, provides significant, world-class, expertise in implantable packaging and interconnect design. We anticipate this new partnership will provide both the innovative design solutions needed but also knowledge of, and relationships with, vendors and suppliers skilled in realizing the designs and assemblies we need.

ISSUES

In general, as expected, 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. We have recently begun the task of fabricating and verifying hardware designs. However, there is still significant work to be done in creating, developing, and fabricating a new class of leadwire and interconnect that can be used to form the network infrastructure; this is still our most challenging technical aspect.

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PROJECT TITLE: Engineering Approaches to Low Vision Rehabilitation

PARTNERS' NAMES AND AFFILIATIONS:

Note: * - Partners that will participate in second period projects (some but not all participated in the first period); # - Partners that completed projects in the first period and were not planned for the second period: Strikethrough -Partners and projects planned for the second period that were eliminated due to budget cuts

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* M. Sodhi (U. of Rhode Island)
* J. Rizzo (Innovative Visual Rehabilitation, VA Med. Ctr, Boston, MA)

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GRANTING NIH INSTITUTE/CENTER: National Eye Institute (NEI)

ABSTRACT

This project applies novel engineering approaches to the problems of low vision rehabilitation. We are building prototype devices based on solid theoretical foundations that, eventually, will become marketable rehabilitation products. The devices, designed and built with the help of our engineering partners, are tested critically using diverse patient populations, with the help of the clinical partners, to determine the effects on function and on the quality of life. We are developing and testing both optical and electronic devices that implement three specific engineering approaches aimed at restoring (at least in part) the important interplay of central (high-resolution) and peripheral (wide-field) vision: multiplexing; dynamic control of display; and image enhancement (the latter was transferred to another grant in the second funding period). In our assessment and testing we emphasize two approaches: a virtual environment for controlled and quantitative testing in the laboratory; and real-world evaluations of the effect and usefulness of the devices and techniques.

STATUS OF RESEARCH AND PARTNERSHIP

The project has two major components: device development and device evaluation. Both components have been progressing extremely well. During the first BRP funding period we published more than 45 peer-reviewed papers in engineering, vision science, and clinical ophthalmic journals. Many of these papers were coauthored with our development (engineering) and evaluation partners. We developed and clinically tested a prism-based device for patients with tunnel vision. We developed and lab tested a head-mounted-display-based device for patients with tunnel vision. Under a SBIR we developed a version of that device for patients with night blindness which was evaluated in the lab and in the street. We developed and tested in a multi center community-based trial a peripheral prism (PP) device for patients with hemianopia. This PP device, developed under another SBIR, is now marketed. A phase 2 SBIR was recently awarded for further development of a second PP design that was conceived during the first BRP funding period. On-road driving studies with the first PP design were completed in Holland and a study with the second PP design is being completed in Belgium. A study of driving with moderate restriction of the visual field was completed in Birmingham, AL. A driving simulator system for testing patients with restricted visual field was developed and pilot tested. A study of patients with hemianopia driving in the simulator is in progress.. A virtual-mall walking simulator was developed and evaluated for testing mobility devices for pedestrians. Two studies evaluating eye movements of patients with low vision while walking on the street were completed. We developed image enhancement in the compression domain to improve video viewing for patients with low vision. We evaluated it first in the JPEG domain with static images and then in the MPEG domain with offline software processing. Then, we developed real-time processing using a standard PC, and are currently setting up to test patients. Studies of eye movements while watching TV, needed for the dynamic control of displays device, have been completed. They showed that most people look at about the same place most of the time, which is needed for that device to be effective. We developed the hardware system and the control software needed for that device and demonstrated its operation. Further studies are planned for the next BRP funding period. A third SBIR was awarded to implement in prototype the in-the-lens telescope concept proposed during the first BRP funding period. A USA patent for that device was awarded. We applied for a second BRP funding period and scored 147. We expect to be funded for that second period.

ISSUES

Despite our success and the outstanding score we received in the review of the renewal application, NEI budget restrictions resulted in a trimming of our budget by about 40%. This substantial reduction means that a number of sub-projects will have to be eliminated completely, and a number of important partners will be removed from the project. Even within the remaining projects we will have to substantially reduce the scope of many efforts. We hope to be able to maintain sufficient progress and coherence in the program to make it possible to bring the second funding period to a successful completion and to develop the remaining aspects under separate funding in future years.

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

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

ABSTRACT

Understanding the mechanisms that underlie abnormalities of electrical conduction in the heart is the key to the development of effective antiarrhythmic therapies. During the last decade, significant progress has been made in imaging electrical excitation waves in the heart using voltage-sensitive fluorescent dyes. However, until recently 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

We completed our work on developing near infrared styryl dyes shifted to the red of the previous generation of styryl dyes by almost 200 nm. A series of excellent dyes, which could be used for optical recording with absorbance and emission wavelengths greater than 650 and 750nm, respectively, were developed (Matiukas et al., 2006). The Loew lab synthesized the dyes, characterized their absorbance and emission spectra in both solution and association with lipid bilayer membrane, and tested the dyes on a voltage-clamped hemispherical bilayer; Dr. Matiukas in the Pertsov lab then characterized them on the heart preparations.

In the most recent studies, our focus was on the developing new chromophores that are tuned to the available inexpensive diode lasers and bright led sources. The most successful of these dyes, JPW-6003, is the most sensitive dye we have ever measured on the hemispherical bilayer apparatus: it showed a $\Delta F/F$ per 100mv of 20% with excitation at 630nm and emission using a long pass of >715nm. In preliminary

experiments with the pig heart preparation, Dr. Matiukas has demonstrated sensitivities as high as 12% for epifluorescence and 20% for transillumination configurations using an inexpensive 658 nm laser for excitation. This is at least twice the sensitivity of di-4-ANEPPS. Furthermore, the new dyes are more persistent and less phototoxic than the previous generation of probes and, because they are red-shifted by 150nm, they allow for recordings from deeper within the tissue. The development of these dyes represents an important step towards in-vivo imaging of electrical activity in the heart.

In collaboration with Dr. Hillman we have started the development of 3D reconstruction procedures and a prototype system for 3D optical cardiac imaging. The system will be built upon a high-resolution 3D optical imaging device called Laminar Optical Tomography (LOT) which was previously developed for rat brain imaging (Hillman et al., 2004). LOT has been shown to effectively image absorption changes in living tissue to depths > 2mm, revealing oximetric changes. We have recently begun to apply LOT to imaging fluorescent contrast, thereby allowing us to image voltage sensitive dyes. We have chosen to begin by imaging trans-mural propagation of excitation through the ventricle of Langendorff-perfused rat heart. LOT's advantage over conventional diffuse optical tomography methods is its ability to make very rapid, non-contact measurements. The z-resolution of LOT scales with depth from ~ 50 microns to ~200 microns over a 2mm range (rat right ventricle ~ 1.5mm, left ~2.5mm). The field of view and penetration depth of LOT imaging is adaptable for future application to thicker tissues such as pig heart.

We recently completed construction of a 2-photon microscopy system, optimized for video-rate in-vivo imaging. Using this system we imaged Di-4-ANEPPS-loaded rat heart tissue. The results of these experiments are very encouraging. Firstly, we were able to visualize Di-4-ANEPPS fluorescence with 2-photon excitation (at 750nm). Secondly, we succeeded in visualizing the stratified fiber layers in the cardiac wall to non-destructively determine their relative angles of rotation (parameters needed for accurate modeling of electrical propagation through the wall).

We hope to capitalize on the success with JPW-6003 dye by modifying the chromophore and varying the sidechains to fully optimize the dyes sensitivity and the staining properties for optical recording of electrical activity deep within cardiac tissue. Sidechains will include ethyl, propyl and hexyl groups on the amino nitrogen. We will also synthesize and test triethylammoniopropyl- and dimethylhydroxyethyammonio-(2-hdroxy)propyl- groups attached to the quinolinium nitrogen. These positively charged headgroups have been successful in producing other highly sensitive dyes.

We will continue to optimize the LOT system for voltage sensitive dye imaging of the heart. We will acquire data on hearts until satisfactory and repeatable data is obtained. We will complete the modeling / simulation step with Dr. Bernus at Syracuse. In collaboration with Dr. Matiukas, we hope to aid development of techniques for rapid two-photon imaging of cardiac excitation. In our system, this would require optimization of emission wavelengths to allow voltage-sensitive discrimination of the fluorescence signal. This would allow high-resolution scans of the heart during pacing for visualization of electrical propagation on a cellular scale at rates of >6000 lines per second.

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 (NIBIB)

ABSTRACT

We are developing 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 is being purchased from Xradia Inc., a leader in developing nondestructive high-resolution x-ray imaging solutions using multi-keV x-rays produced by both laboratory and synchrotron x-ray sources. 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 FY02 and we have made excellent progress during this past year. In October 2005, we placed the order with Xradia for the Phase I implementation of the Transmission X-ray Microscope (TXM) that forms the basis of our bioimaging facility and finalized the optical design in January 2006. In addition, with the arrival of the second year

funding, we placed the order for Phase II of the TXM implementation in June 2006. The timely placing of this second order will allow Xradia to deliver the TXM complete with the Phase I and II implementations in September 2006. The instrument will then be capable of both 2D and 3D tomographic imaging in absorption contrast at 20 or 60 nm resolution in the photon energy range between 5 and 14 keV. It should be noted that the Phase III implementation of the instrument is scheduled for FY03 of this program in which an additional elliptical condenser, zone plate, phase ring and software will be implemented for phase contrast imaging at 8 keV.

We will start commissioning the instrument with the start of the new SSRL run in October 2006. We expect to achieve sub-60 nm spatial resolution images of resolution test patterns and first bone samples in 2D and 3D by the end of 2006 using absorption contrast. The spatial resolution will be improved to 20 nm during the first quarter of 2007 by using the zone plate in third order to obtain the images. This will also be the time when biological imaging, beyond what was needed for commissioning, will start. This schedule will allow the biological researchers to become fully versed with the operation of the TXM as well as making available significant amounts of imaging time. The funding for the biological part of the program, which will investigate the effect of weightlessness on the nanostructure of bone, has started this year.

ISSUES

None.

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PROJECT TITLE: Nanotechnology for the Structural Interrogation of DNA

PARTNERS' NAMES AND AFFILIATIONS:

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GRANTING NIH INSTITUTE/CENTER: National Human Genome Research Institute (NHGRI)

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

In our second year of this project we have made significant advances 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. In the past report we have shown that DNA sequencing via transverse transport can be accomplished by comparing the *distributions* of electrical currents for the different bases while they translocate through a nanopore. In that work we have also shown that a transverse field of the same magnitude as the one that drives the current is enough to stabilize the DNA inside the pore thus making the current distributions distinguishable. In the past months we have checked that such results are robust with respect to the transverse field, i.e. we have checked that even small fields are enough to control the DNA dynamics. As

in our previous work we have combined molecular dynamics simulations with quantum transport calculations [J. Lagerqvist, M. Zwolak and M. Di Ventra "Fast DNA sequencing via transverse electronic transport", *Nano Letters* 6, 779 (2006).]. We have found that even a bias of 0.1V - i.e. one order of magnitude smaller than the one previously employed, across the electrodes where electrical current flows is enough to control the DNA dynamics and make the base distributions distinguishable. Most importantly, the error as a function of number of independent measurements with which these bases can be distinguished remains practically the same even at this small voltage.

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. We carried out extensive molecular dynamics calculations to investigate the ionic conduction in a nanopore of about 2 nm in diameter in the presence of single-stranded DNA (ssDNA), with the ultimate goal of determining the difference in ionic current by single base variation along the DNA. We have investigated the mechanism of the ionic conduction in nanopores in the presence of short single-stranded DNA oligomer and the resulting current difference. This is compared with the experimental work using a-hemolysin pores, where a reduced ionic current from 120 pA for open channel to 6 pA and 15-20 pA is observed in the presence of poly[C] and poly[A], respectively. Through the molecular dynamics calculation, we found the co-ions to be strongly depleted in the nanopore due to strong electrostatic repulsion by DNA, and thus the current conduction through the nanopore is mainly determined by the counter ions. The DNA-counter-ion interaction is also strongly enhanced due to nanopore confinement with the counter-ions strongly attracted to the ssDNA phosphate groups, resulting in a long resident time of several nanoseconds, and a mobility orders of magnitude slower than in bulk solution. Based on this study, we proposed a mechanism for the ionic current conduction through nanopore in the presence of DNA, in which the counter-ions reside with a phosphate group of DNA and then occasionally hop to the next site on the ssDNA backbone, much in the same way as the hopping of positively charged holes in a semiconductor. Using the calculated ionic diffusion coefficient and the concentration from the molecular dynamics, and the Nernst-Planck equation for flux, we determined the ionic current through the nanopore. In particular, the magnitude of the current agrees with experiment and shows that ionic current in the presence of poly[A] is about 3 times larger than poly[C], as in experiment.A manuscript describing the work has been submitted for publication [S. T. Cui, "Counterion-Hopping along the Backbone of Single-Stranded DNA in Nanometer Pores for Current Conduction", *Phys. Rev. Lett*. submitted (2006)].

The UNC group has been developing techniques for forming nanopores silicon nitride nanoscale membranes. We have two different fabrication strategies for forming nanopores in the silicon nitride membranes that results in pores with diameters in the range of 2-5 nm with film thicknesses of either ≈ 10 nm or ≈ 100 nm. DNA translocation events have been observed for the thin membranes. Computer codes have been developed for analyzing translocation events. We have also been studying the effects of surface modification of nanopores. We are presently establishing techniques for performing surface modification and quantifying the quality of the modification.

Baddorf, Jesse (ORNL) and Weitering (UTK) continue to work on development of nanoscale electrodes for probing DNA. The ORNL group is using electrodes consisting of carbon nanotubes (CNT) while UTK is growing metal silicide nanowires. Both approaches have challenges regarding their interface with nanochannels. These fabrication issues are being addressed. The group of Feldman at Vanderbilt University has been assisting ORNL with nanofabrication issues and Dickinson's group at WSU continues developing AFM based chemical mechanical polishing techniques for nanochannel fabrication.

ISSUES

None

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PROJECT TITLE: Optical Molecular Imaging of Cancer

PARTNERS' NAMES AND AFFILIATIONS:

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GRANTING NIH INSTITUTION/CENTER: National Cancer Institute (NCI)

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 EGFR, MMP, telomerase and v integrin; (2) to identify promising new biomarkers 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 non invasively 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

During year 2 we made progress of various aims of the proposed research:

Aim 1 Year 2: In the area of optically active contrast agents, we developed gold and silver nanoparticles of spherical and cylindrical geometries. The results of this development provide a potential for multi-spectral imaging in tissue environment with sub-nanomolar concentration. For their bio-conjugation, we are working towards development of various coatings including bio-polymer coatings such as oligonucleotides to improve stability and ability to specifically link variety of biomolecules ranging from ligands, peptides and antibodies. For development of quantum dot based contrast agent, we analyzed various coating approaches including MPA and other amphiphilic biopolymers for stability against aggregation and photo bleaching. We also conjugated novel targeting agents such as aptamers to quantum dots and demonstrated specific targeting of cancer antigens such as PSMA in model systems.

Aim 2 Year 2: For the specific aim of designing new imaging targets, we made significant development in combining combinatorial phage display libraries with gold nanoparticles. This allows for creating hybrid structures, which has ability to screen variety of ligands on cell surfaces as well as sensitive reporting using surface enhanced plasmon resonance properties of nanoparticles. We demonstrated its application in screening and imaging using model cancer cell lines. Future work will develop new targeting ligands for various cancer-associated antigens. In the area of development of genomic approaches for developing novel imaging targets, we generated SAGE tag libraries using biopsy samples from LEEP procedure. This approach identified five new genes, which are expressed only in cervical tissue.

Aim 3 Year 2: We characterized gold nanoparticle-anti EGFR conjugates for specific targeting of cancer cells and tissue model phantoms. We analyzed the specific spectral characteristics of metal nanoparticles contrast upon targeting EGFR receptors. We developed dual reporting contrast agents which combines reflectance and fluorescence imaging in a single contrast agent. The results of EGFR receptor targeting in cervical biopsy samples show significant contrast enhancement for metal nanoparticles as compared to fluorescence imaging. These results demonstrate the effect of surface plasmon enhancement obtained with nanoparticles based contrast agents. In addition, we also systematically studied the delivery of nanoparticles of various size ranges using variety of defined nanoparticles sizes and various permeation enhancers. In this area, we are having some challenges due to large variability in top surface layer of mucosal tissue and its protein contents specifically keratin layers. In future we will explore minimal invasive methods such as microneedles along with permeation enhancers to overcome some of the barriers in this area.

Aim 4 and Aim 5 Year 2: For the specific aim of development of imaging systems we significantly advanced the development and validation of three different imaging systems. In first case, we designed, developed and validated imaging in model systems with needle biopsy imaging system. This system will allow imaging of the microenvironment in tumors and other inaccessible places. In second case, we developed a multi-spectral diffuse imaging system to image lesions in head and neck anatomical sites. This system has capabilities to provide multi-spectral imaging with a wide field of view. In a third case, we developed dual confocal imaging systems which allow imaging of reflectance and fluorescence in tissue biopsies. In addition to these systems, we constructed fiber optical fluorescence imaging system which will allow imaging in the lung cavity. These systems along with development of contrast agents will provide various potential opportunities to investigate cancer biology with high specificity and sensitivity.

ISSUES

The scientific issue with contrast agents and their delivery is the variability in delivery efficiency across different tissue samples. We attribute this to differences in the structure and protein content, in particular keratinization, of top tissue layers. This has been the most significant factor in limiting the repeatable delivery of contrast agents. In this area, we are exploring the use of minimal invasive tools such as electroporation and microneedles to enhance the delivery process. In addition, stability of bio-conjugates in tissue environment has been cause of some concerns. In this area we are developing chemical conjugated chemistries to overcome this challenge.

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PROJECT TITLE: Spinal Control of Locomotion: Studies and Applications

PARTNERS' NAMES AND AFFILIATIOS:

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

ABSTRACT

The goals of this project are (1) to perform a comprehensive multidisciplinary study of neural mechanisms in the mammalian spinal cord responsible for generation of the locomotor pattern and control of locomotion and (2) to find optimal strategies for restoring locomotor function after spinal cord injuries. In this project, two comprehensive databases will be created based on experimental studies of fictive locomotion in the decerebrate cat and on biomechanical studies of freely moving uninjured cats and spinal cats. These databases will be used for the development of (1) a computational model of neural circuitry of the spinal cord responsible for generation and control of the locomotor pattern and (2) a neuro-musculo-skeletal model of cat's locomotion. Special quantitative biomechanical criteria will be developed for evaluation of locomotor capabilities of spinal cats during and after implementation of different treatments for restoring the locomotor function. The computational models and biomechanical criteria developed will provide guidance for the applied treatments and evaluation of their results. Different strategies for the restoration of locomotor capabilities based on the combination of locomotor training on a treadmill with phase-dependent electrical stimulation of the selected sensory afferents will be implemented and investigated.

STATUS OF RESEARCH AND PARTNERSHIP

The project started two months ago. During this short period, there was some progress in each group.

Dr McCrea has performed a series of experiments on decerebrate immobilized cats with fictive locomotion evoked by midbrain stimulation and bilateral recordings of activity (ENGs) of major flexor and extensor hind limb motoneurons. The results obtained have confirmed the maintenance of the phase of locomotor oscillations during deletions of motoneuron activities even when the contralateral locomotor activity is absent. These results provide additional support for the two-level model of the locomotor central pattern generator (CPG) incorporated in our computational model of the spinal cord circuitry (developed at Drexel University by Dr. Rybak group).

Dr. Rybak has developed a new version of the locomotor rhythm generator that includes a homogenous population of directly coupled excitatory neurons that is split into two "half-centers" inhibiting each other via inhibitory interneuron populations. The endogenous rhythmogenic properties of

single neurons (modeled in the Hodgkin-Huxley style) are based on the persistent (slow inactivating) sodium current. The model can generate a realistic range of locomotor cycle periods. An increase in the excitatory drive to the half-centers decreases the cycle period. The durations of the flexor and extensor phases replicate the wide ranges seen in real locomotion and can be adjusted by regulating intrinsic neuronal properties, mutual inhibition and tonic excitatory drives to the half-centers. In accord with the in vitro observations in rats, blockade of synaptic inhibition in the model results in synchronized oscillations of flexor and extensor activities. This model has been incorporated into our model of spinal circuitry in which the CPG is comprised by separate rhythm generator and pattern formation networks. The two-level CPG allows for independent control of locomotor phase timing and the degree of motoneuron recruitment. The two-level CPG architecture allows the model to reproduce a number of experimental phenomena obtained in the Dr. McCrea's laboratory during studies of fictive locomotion in cats. These include (a) realistic motoneuron firing patterns, (b) spontaneous deletions of rhythmic activity during locomotion, and (c) perturbations of motoneuron timing and recruitment produced by sensory feedback from hind limb muscle and cutaneous afferents. The model provides a basis for functional identification of spinal interneurons involved in generation and control of the locomotor pattern.

Dr. Prilutsky has started parameter identification for the musculo-skeletal model of cat hind limbs. The purpose of this procedure is to find a set of parameters for a forward dynamics musculo-skeletal model that can provide a close match between the computed and experimentally recorded biomechanical characteristics of cat locomotion. The forward dynamics musculo-skeletal model of cat hind limbs will be initially driven by the patterns of muscle activation recorded experimentally and used as an input to the model. To reduce possible errors in model performance, the values of parameters will be optimized by minimizing a cost function that represents a calculated generalized difference between the characteristics of movement generated by the model and the same characteristics recorded experimentally from the freely walking, uninjured cat. Kinematics and kinetics of walking in the uninjured cat necessary for realization of the above procedure have been obtained using inverse dynamics methods. A simulated annealing optimization algorithm (Corana et al. 1987) has been successfully tested using a 2D function with several dozens of local optima.

Dr Lemay has initiated the development of a split-belt treadmill, instrumented with a force plated. This treadmill will allow us to measure ground reaction forces in spinal cats trained to locomote on a treadmill and to compare the kinetics of gait in spinal cats with the same characteristics obtained at Georgia Institute of Technology (Dr. Prilutsky laboratory) on the same animals before spinalization. A number of nerve cuff electrodes have been built for implantation into cats that will be initially trained in the Dr. Prilutsky laboratory. These animals should provide further data on the effects of afferent stimulation on the gait of spinal cats trained to locomote on a treadmill. The results of preliminary experiments on one animal have shown that stimulation of the sural nerve can enhance the swing phase of gait, while stimulation of the tibial nerve during the stance phase can prolong this phase. These results match well with the data of Dr. McCrea obtained on fictive locomotion cat preparations and have also been predicted by Dr. Rybak's neural model of the spinal cord circuitry.

ISSUES

The project has just started and no issues of concern have been identified so far. Subcontracts to University of Manitoba (Canada) and Georgia Institute of Technology (Atlanta, GA) have been put in place. There is some delay with the hiring of two key investigators. They both are coming and will be able to start working within this month (July). Interactions between the four groups involved in the project are running efficiently.

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PROJECT TITLE: High Frequency Ultrasound Arrays for Intracardiac Imaging

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

ABSTRACT

Cardiac dysrhythmias of atrial fibrillation and flutter affect about 2.4 million Americans with approximately 160,000 new cases in the US alone every year. Electrophysiological (EP) and radiofrequency ablation (RFA) procedures to treat cardiac arrhythmias are among the most prolonged and detailed due to the difficulties in the accurate guidance of catheter based diagnostic and therapeutic devices within the cardiovascular system. Although fluoroscopy is still the gold standard imaging tool, it is well recognized that with longer and more challenging procedures new and more effective real time spatial mapping tools are vitally needed to improve clinical outcomes and to reduce the fluoroscopic radiation doses to which patients are currently exposed.

We have proposed to design, develop and test a family of 2D and real-time 3D intracardiac echocardiography (ICE) imaging devices which, at 10-15MHz operating frequency, can 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 imaging devices are integrated with EP sense electrodes and RF ablation electrodes so that they can visualize an intracardiac target lesion, anatomically monitor the ablation procedure and map the distribution of temperature during RF delivery. As an addition to the multi-functional nature of the catheters, the EP sense electrodes provide a means of 3D positional information when the devices are used together with available electroanatomical mapping techniques.

STATUS OF RESEARCH AND PARTNERSHIP

Our partnership has successfully tested *in vivo* two of the three proposed designs and has significant laboratory experience with bench prototypes of the third design type. The first device, the 9F 64-element EP-enabled HockeyStick (HS), has been tested in two design formats in porcine models of pacing and ablation and has been found to provide high quality RFA resistant imaging of intracardiac anatomy. As well, the HS device has been used successfully in a porcine animal model as an integrated 3D mapping catheter with a commercially available electroanatomical mapping system (ESI, NavX). Two HS imaging catheter designs, with various placements and number of EP sensor electrodes, have been spatially tracked in 3D endocardial volume mapping with 1mm spatial precision within the porcine cardiovascular anatomy in real time.

Our second EP-ICE combination catheter design, the "MicroLinear" (ML), is equipped with EP sensor bands near the distal tip, and as well, a 14 MHz, 24 element forward looking ultrasound array. The first catheter prototypes, built with a PZT ceramic ultrasound array, have produced high quality, high frame rate images in 2 porcine animal models. Although this device is designed for imaging in the 3 to 10mm range, it performs well to 20mm and beyond. The first ML devices tested *in vivo* have been built as 9F catheters shafts with a 15F experimental tip that houses the high frequency imaging array along with side ports to support a thermocouple equipped wire and a postioning/stablization wire to allow direct site positioning and monitoring of tissue during RF ablation procedures. Both the ablation catheter and tissue ablation site could be easily observed in real time during the RFA procedure. The second generation ML design is in process to augment steering, reduce the tip to the 9F target size, and add a tip ablation electrode. A high intensity focused ultrasound test is also planned with the ML device design to test the feasibility of a micro-HIFU approach in intracardiac ablation therapy. High frequency ultrasound data has been collected from the ML catheter and is undergoing analysis for tissue strain due to the heating of ablation. A second array design for the ML catheter, to be built of capacitive micromachined ultrasonic transducer (cMUT) elements, is underway that is targeted for first testing in the Fall of 2006.
The third EP-ICE device, the "Ring Array" catheter, is designed with a cMUT based array, and has been built first as a bench top prototype array which has successfully produced high quality 3D images of stent struts and wire targets. The second generation of this device, which will integrate custom array electronics with the circular array is underway and testing of this next assembly generation device is expected in late 2006.

In the thorough preparation necessary to acquire FDA approval for an investigational device exemption (IDE) for the HockeyStick EP-ICE catheter clinical trial, the partnership has undertaken very extensive testing and validation efforts to satisfy all safety standards of the highest level. In this effort, we have built and tested 35 fully operational HS catheters, as well as 24 quasi-functional (reduced imaging capability) and 83 mechanical catheters. The partnership met and received valuable feedback from a March 2006 Pre-IDE meeting with the FDA. We are in the final phase in the IDE submission process and expect to begin the HS clinical trial in the Fall of 2006.

ISSUES

With the vigorous pusuit of the HS catheter IDE over the last year, the partnership has expended considerable resources for HS building and testing that will limit the funds available for a self supporting extension, so we do plan to submit a competing renewal for the January submission date in the reissued RFA for BRP's PAR-06-459 before the end of our 5th year of funding .

A technical paper describing laboratory testing of the Ring Array design was selected as an award finalist at the IEEE Ultrasound Symposium in Rotterdam, Holland in September 2005. This work demonstrated clear visualization of *in vitro* intracardiac stents with a 2mm diameter, 64 element annulus, 8 MHz cMUT array which was also capable of 19MHz performance. A clinically oriented paper was presented at the 2005 AHA meeting in Dallas describing our experience with the HS catheter *in vivo* with excellent performance in the modalities of strain rate, tissue velocity, color flow, and tissue ablation guidance.

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PROJECT TITLE: High-speed Depth-Resolved Imaging of Cardiac Electrophysiology

PARTNERS' NAMES AND AFFILIATIONS:

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

ABSTRACT

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

The long-term goal of this Bioengineering Research Partnership (BRP) is to develop a **High-Speed, Depth-Resolved Imager** (**HSDRI**) to map electrical activity or intracellular free Ca^{2+} transients inside the myocardium of perfused hearts. The partnership consists of 3 groups. Dr. Guy Salama (PI at the University of Pittsburgh) will administer the BRP, develop the instrument and apply the new technology to problems in cardiac electrophysiology, that remain unresolved due to a lack of 3-D information. Drs. Alan Waggoner (at Carnegie-Mellon University, director of the Molecular Biosensor and Imaging Center (MBIC) and Lauren Ernst will develop optical probes (voltage-sensitive, Ca^{2+} and K^{+} indicator dyes) with long excitation and emission wavelengths to improve tissue penetration and reduce light scattering from the myocardium. Dr. Fred Lanni (at MBIC) will provide the theoretical and engineering expertise to develop and refine the HSDRI. The 3 groups will work in parallel.

Aim 1 (Salama and Lanni): To developed and test the best possible HSDRI system. We have now built and spinning disk confocal imager (DSU) (some components from Olympus, our optics and software) in combination with a high frame rate (10,000 frames /s) CMOS camera (SciMedia USA)

Aim 2: Drs. Waggoner and Ernst have synthesized new longer wavelength fluorescent dyes to monitor action potentials (APs) and Dr. Salama has tested, analyzed their spectral and response characteristics in heart muscle.

Aim 3 (Salama, Choi and Lanni): Software has been developed to drive the **HSDRI**, analyze APs and map electrical activity in 3-D. Depth-resolved maps of activation, repolarization and AP durations will be used to investigate 2 topics in cardiac electrophysiology, where measurements in 3-D are essential to elucidate fundamental concepts.

A) We will investigate the factors that modify electrical coupling (time-delay or block) between Purkinje fibers (**P**), Transitional (**T**) and Ventricular (**V**) cells to elucidate the **role of PV junctions** in the initiation and maintenance of arrhythmias.

B) **Impulse propagation across the atrio-ventricular node** (AVN) has been difficult to trace because of the complex 3-D structure of the node and the small region of compact cells. Activation maps of the AVN in 3-D will help us answer basic questions regarding the precise inputs to the node (fast and slow pathways), mechanisms of AVN reentry, Wenckebach periodicity and Wolf-Parkinson syndrome. Fast, depth-resolved images of voltage and Ca^{2+} are a powerful new tool that will have a wide range of applications in cardiac electrophysiology and can be extended to neuronal networks and other organ systems.

STATUS OF RESEARCH AND PARTNERSHIP:

In the first 3 years of this BRP, we put together an instrument to map electrical activity of the heart in 3-dimensions. 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 a DSU. We have building a 3-D imager for large fields of view and have used it map AP propagation inside the wall of a guinea pig heart (1x1 cm^2) at a depth of 300 ± 50 μm and with the high speed CMOS camera the signal to noise ration was ~ 40/1 at 1K frames/s. A manuscript will appear on a Monte Carlo simulation of the optical depth resolution of the apparatus, with an experimental verification of the theoretical calculations.[1]

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. Towards that end, Drs. Alan Waggoner and Lauren Ernst synthesized new 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. A set of 5 new probes were developed that can be excited at ~ 700 nm and emit at ~ 850 nm. One of these dyes, Pittsburgh I (PGHI) has been found to have twice the sensitivity to voltage compared to the best currently available probe (di-4-ANEPPS) and can be excited at 690 nm with a peak emission at 850 nm. Pharmacological effects, stability, phototoxicity and the 'Action Spectra" (voltage-dependent spectral changes) of these new dyes were measured and reported last year[2] and a second paper describing the synthesis of these dyes is about to be submitted.[3]

A new class of dyes with PEG linkers designed to improve dye retention and binding to the cellular membrane are currently being tested under different staining conditions by varying the vehicle to maximize the voltage signals that can be obtained at long wavelengths. We are also attempting to make a new class to dyes that will bind to the cell membrane with an extracellular moiety that reports on the extracellular K^+ concentrations in the external milieu.

To improve our discrimination between ventricular myocytes (V) and Purkinje fibers (P), we collaborated with Dr. Michael Kotlikoff at Cornell University to use genetically encoded Ca^{2+} sensors that are expressed selectively in V or P cells. We recently reported the characteristics of a molecularly engineered mouse with the GCaMP2 Ca^{2+} probe encoded in ventricular myocytes[4] and have used embryonic myocytes from these mice to repair a myocardial infarct.[5] In collaboration with Dr. Kotlikoff, we are studying another mouse with the probe expressed exclusively in the Purkinje network. We will plan to combine our 3-D HSDRI system to map Cai transients across the Purkinje network and measure the time delays between P and V cells loaded with Rhod-2. Ca_i will be mapped in 3-D to resolve PV delays during antegrade and retrograde conduction, normoxic and ischemic in paced and during arrhythmias.

ISSUES

As predicted by the reviewers the proposal has the potential of discovering new optical probes and instrumentation that might be worthy of patent submissions and commercialization. New probes have been sent gratis to various laboratories so that they may compare them with their current probes of membrane potential to allow us to better evaluate the performance of these dyes. Several labs have indicated that they prefer our dyes and would like to use them on a regular basis. An important issue that we need to resolve is how to provide the new compounds to other investigators and to best serve the scientific community. The partnership has worked well. The three research groups come together every 2 weeks for face-to-face discussions of overall progress and to stay focused on our goals. The project is on schedule.

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

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Jeffrey Ojemann and Eric Leuthardt (Dept Neurosurgery, Univ Washington, Seattle, WA)

GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institute of Neurological Disorders and Stroke (NINDS)

ABSTRACT

Signals from the brain can provide a new communication channel, a brain-computer interface (BCI), for people with severe neuromuscular disorders such as amyotrophic lateral sclerosis (ALS), brainstem stroke, cerebral palsy, and spinal cord injury. BCI technology can allow people who are completely paralyzed, or "locked in," to express wishes to caregivers, use word processing programs, access the Internet, or even operate neuroprostheses.

Up until 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 people 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 BRP project are: (1) to develop a flexible general-purpose BCI system that can incorporate any of the relevant signals, analyses, and operating formats and that 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 that has clear practical value for those with motor disabilities.

STATUS OF RESEARCH AND PARTNERSHIP

The past year has seen substantial progress. The program continues to focus on: development and dissemination of BCI2000, our general-purpose BCI system; optimizing use of this system to enable people with and without disabilities to use EEG-based BCI methods (sensorimotor (i.e., mu and beta) rhythms and the P300 evoked potential) and related electrocorticographic(ECoG)-based BCI methods; and clinical implementation and validation of this BCI technology. Major progress includes:

(1) Further improvements and extensions of BCI2000. These include: interfacing the system with off-the-shelf communication aids (such as predictive spelling programs); and developing related software tools (e.g., the offline analysis program MARIO and a new BCI2000 plug-in for real-time visualization of brain function called SIGFRIED (SIGnal modeling For Real-time Identification and Event Detection). To date, BCI2000 has been adopted by more than 80 laboratories around the world and is rapidly becoming the standard in the field. It is supported by a very energetic user group and an active website.

(2) Further improvements in EEG-based multidimensional and sequential movement control. With these refinements, trained users can now master two-dimensional movement to a goal (i.e., reach) followed by selection of the goal (i.e., grasp). Two independent EEG control signals move a cursor to one of multiple possible targets in two dimensions, and a third selects or rejects the target. In studies to date, users, including those with spinal cord injury, achieve >90% accuracy.

(3) The Atlanta partner continues to provide BCI2000 software adaptations including improvements to the P300 program and the 3-D application. This partner also provided the initial programming and testing for BCI2000-based SSVEP control.

(4) Together with the Tuebingen partner, we are implementing and evaluating clinical use of the laboratory-proven BCI systems described above. To do this, we have significantly reduced the complexity of the necessary hardware and BCI2000 configuration to produce a BCI2000-based home system. We have begun testing this system for everyday use by people severely disabled by ALS. The first such user is a NIH-funded scientist with ALS who can only move his eyes. He has had the BCI home system for 5 months and reports that he much prefers it to his eye-gaze communication system. He now uses the BCI system 6-8 hrs/day for e-mail and other purposes.

(5) With our partners at Washington University in St. Louis and the University of Washington in Seattle, we continue to explore the BCI use of electrocorticographic (ECoG) activity recorded from the cortical surface. We recently completed a study that demonstrated that ECoG can be used to decode position and velocity of two-dimensional hand movements, and showed that the accuracy of this decoding is comparable to that previously described for signals recorded using intracortical microelectrodes. We also completed a study that demonstrated that ECoG recorded can support two-dimensional movement control.

(6) Finally, to continue to support and develop the field of BCI research, we have hosted the 3rd International BCI Meeting, edited a special BCI issue of the IEEE Transactions of Neural Systems and Rehabilitation comprised of 31 peer-reviewed articles from the meeting, and hosted the first BCI2000 Workshop.

ISSUES

The work of the BRP is progressing very well. The most critical issues concern the appropriate allotment of the Partnership's time and effort among the important opportunities and responsibilities now presenting themselves. Chief among these are: the continued dissemination and support of the general-purpose BCI system BCI2000; the validation of BCI clinical applications and their dissemination to the user populations most in need of them; and continued exploration and progressive clinical implementation of EEG/ECoG-based BCI technology.

The continued maintenance and support of the BCI2000 software now in use by more than 80 research laboratories around the world is the purpose of a newly funded RO1 application. The development of an appropriate mechanism for ensuring that BCI home systems can be provided (along with technical support) to those who most need them is the subject of current discussions. It is likely that a non-profit entity will eventually be established to serve this function. The primary work of the BRP will continue to focus on improvement of EEG-based BCI communication and control, and on concurrent development of related ECoG-based BCI methods, which, like EEG-based methods, will eventually come to clinical implementation and validation.

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PROJECT TITLE: Smart Substrates for a New Generation of Implants

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

ABSTRACT

Infection following joint arthroplasty is a devastating complication with immense financial and psychological costs. Effective countermeasures to prevent osteolysis and infection include the use of body exhaust systems, laminar airflow and prophylactic antibiotics. Despite these measures, deep infection still occurs in 1-5 percent of joint replacements. The goal of this project is to engineer a "smart" prosthesis that addresses these problems. The scope of this highly integrated research requires collaboration across traditional academic boundaries. The proposed study will be spearheaded by investigators at two leading academic centers with strong emphasis on bioengineering, dental and orthopaedic research. The first objective will be to engineer a Ti surface that is osteogenic due to the presence of the adhesion peptide RGD. The broad spectrum antibiotic tigecyclin will be tethered to the metal using the adhesion peptide stem to yield a surface that is both osteogenic and anti-bacterial. We will measure the efficacy of the tigecyclin against a bacterial infection and determine the osteogenic potential of the tethered RGD peptide. In Specific Aim 2, a sol-gel film will be generated on the modified Ti surface that will release antibiotics and BMP-2. The osteogenic response and the release of the antibiotic linezolid from the sol-gel will be determined. In Specific Aim 3, we will engineer a prosthesis using these modified surfaces for in vivo testing in a dog hip infected with S. aureus. Immunogenicity, infection eradication, and bone formation/osseointegration will be evaluated. Following twelve weeks of recovery, femora will be recovered and analyzed using physical, optical, and histological techniques. Outcomes from this study will provide information for engineering of orthopaedic and dental implants that can be tested at the clinical level.

STATUS OF RESEARCH AND PARTNERSHIP

The major objectives of the work performed in the 01 year of the grant addressed the goals of the first two Specific Aims.

Chemistry: The antibiotic of choice is tigecycline which has a similar structure to tetracycline, targets inhibition of protein synthesis, and provides broad gram negative and gram positive coverage. As a first step towards broad coverage, we have tethered vancomycin, ceftriaxone, tetracycline, and gentamicin to Ti through aminopropylation, aminoethoxyacetate linker addition, and coupling of the antibiotics. Additionally we have been synthesizing tigeyclin-like derivitives of minocycline for characterization.

Generation of a Titanium Surface for Antibiotic Bonding : A major goal of this proposal is to prepare and functionalize titanium surfaces for subsequent studies of bacterial and osteoblastic adhesion. To prevent confounding results due to surface roughness and surface chemistry, we have developed methods to prepare and oxidize Ti to attach self assembled silane monolayers for attachment of peptides and antibiotics. Initially, extensive efforts (6 months) were made to polish Ti alloy disks (supplied by Stryker) using custom-designed holders to enable

6 disks to be simultaneously polished using successively finer diamond polishing materials. After passivation, surface roughness could not be reduced below about a micron by AFM. Secondly, smooth titanium surfaces were eventually prepared by using a thermal evaporator and resulted in a rms roughness of only 1 to 2 nm's. The passivation steps also needed to be refined. Four methods are being explored including nitric acid, Soxhletting in chloroform, standard piranha etching, and modified piranha etching. The nitric acid treatment had little effect on the Ti. The Soxhletting method appeared to remove surface contaminants as noted by a decrease in contact angle from 55^{O} to 35^{O}, with the drawback that only a few samples can be prepared coupled with extensive set-up time. The standard Piranha etch was too aggressive and effectively stripped off the Ti film entirely. A modified Piranha, which involved a cold, 5 min incubation of the samples in the Piranha etch, was most successful.

Microbiocidal Properties of the Antibiotic Modified Surface. Modified surfaces were prepared after passivation using the standard Piranha protocol and solid state coupling of aminpropyltriethoxysilane, aminoethoxyethylacetate and antibiotic. Antibiotic-derivatized surfaces were incubated with dimethylformamide followed by PBS for 24 h to remove unreacted chemicals and/or adhered antibiotic. These surfaces were then incubated with *S. aureus*, our model gram positive organism and *E. coli*, our model gram negative organism. After a 24 h *S. aureus (*gram positive*)* challenge, colonization was decreased with surface-bound vancomycin, gentamicin, and the amino terminal linkage of ceftriaxone; the carboxy-tethered ceftriaxone and tetracycline were not active. *E. coli* (gram-negative) showed colonization on the vancomycin-modified nails, with different degrees of inhibition of colonization with the other antibiotics with gentamicin being the most potent. These experiments demonstrate that a wide range of antibiotics can be bonded to the nail surface and remain active, allowing for broad coverage against polymicrobial infection. In parallel experiment, mixed RGD/vancomycin surfaces were prepared. As an initial step in their characterization, these mixed surfaces were incubated with S. aureus and bacterial colonization assessed. As these surfaces display a stoichastic distribution of antibiotic and RGD in a 50:50 ratio, bacterial colonization was only moderated inhibited. Importantly, as expected, the pure RGD surface was not as avidly colonized as the control derivatized surface.

Sol Gel Studies. The objective of the second Specific Aim is to develop and evaluate a biologically active Ti implant surface that permits short-term, local delivery of antibiotics (linezolid) while enhancing osteogenesis. During the initial funding period, we have been fabricating a thin sol-gel film on a Ti-alloy substrate using an acid/base catalyzed hydrolysis reaction that permits subsequent loading of the sol-gel film with the antibiotic. We have also varied the synthesis parameters to determine their effects on degradation and release properties of the sol-gel films in vitro. The sol-gel process involved preparation of a liquid sol as a first step. Tetraethylorthosilane (TEOS), deionized water (DI), ethanol, and 1N HCl (DI:HCl:TEOS molar ratio=5:001:1 and ethanol/TEOS vol. ratio=2) were mixed and stirred to form an acid-catalyzed sol (pH 1.8) and 0.1 M NH_4OH was added to obtain acid-base catalyzed sols with pH varying in the range from 3 to 7. As the pH increased from 3 to 7, the time to gelation decreased from 10,000 to 10 minutes. Sols with nominal antibiotic concentrations of 0, 5, 10, and 20% by weight were prepared by adding aqueous solutions of the drug, and antibiotic was successfully incorporated into acid-base catalyzed sols with pH equal or below 4.5; above pH 4.5, antibiotic precipitated. These sols were then used to deposit a single-layer film on cleaned and passivated Ti-alloy samples (0.5x13x25 mm). The in vitro assay of the degradation and release properties of the acid-base catalyzed sol-gel films was performed in PBS (pH 7.4) at 37^{0}C with daily solution exchange. Film degradation was monitored by measuring the weight loss of the film, and released antibiotic was measured spectrophotometrically. Both time-dependency and load-dependency of degradation were measured. Films with the higher load of 10 and 20% showed a sustained drug release up to 4 days of immersion. In comparison, films with the 5%-load showed a slower release, which continued up to 6 days. In addition, the daily released concentrations of the drug exceeded the MIC of the antibiotic against S. aureus. This initial study suggests that is possible to apply a thin controlled release antibacterial acid-base catalyzed sol-gel film on a Ti-alloy substrate. It was also demonstrated that a high drug load, up to 20%, can be incorporated in the films and then released in a controlled manner.

ISSUES

Scientific: We have been exploring additional ways of optimizing derivatization of the surface while minimizing corrosion and roughness. We will continue to develop the chemistry for attachment of the antibiotic tigecyclin/9-amino-minocycline to the Ti alloy surface. This attachment has been a problem and it may be necessary to switch to a mixture of the other antibiotics described in this report to attain the desired broad-spectrum coverage. Once the bactericidal capabilities of surfaces with random distributions of antibiotics are elucidated, we will continue with the plan of designing nanoscale distributions of antibiotics and ligands to optimize the osseointegrative and bactericidal properties of the modified surface.

Administrative: No issues have arisen this year.

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PROJECT TITLE: Transcriptome and Proteome Mapping of the Mouse Brain

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

ABSTRACT

Our goal is to create genome-scale maps of gene expression in the mouse brain using a new approach called voxelation. This method employs high-throughput analysis of spatially registered voxels (cubes) to produce multiple volumetric maps of gene expression analogous to the images reconstructed in biomedical imaging systems, such as CT, PET and MRI. We are mapping both the transcriptome and the proteome.

STATUS OF RESEARCH AND PARTNERSHIP

We have reconstructed 2-dimensional images of gene expression for 20,000 genes in a coronal slice of the mouse brain using microarrays in combination with voxelation at a resolution of 1mm. Good reliability of the microarray results was confirmed using multiple replicates, subsequent quantitative RT-PCR and publicly available in situ hybridization data. Clustering analysis identified known and novel genes with expression patterns localized to defined substructures within the brain. In addition, genes with unexpected patterns were identified. Using LC-FTICR mass spectrometry, we have also obtained expression patterns at 1 mm resolution for hundreds of proteins (~600) from the same coronal section employed for the transcript studies. Comparison of the proteomic voxelation data with the microarray voxelation and in situ data revealed good agreement between these very different expression maps. The genome-scale maps of gene expression obtained using voxelation will be a useful tool for the neurogenomics community, while providing valuable insights into the molecular architecture of the mammalian brain.

ISSUES

None

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PROJECT TITLE: High Field MRI: Limitations and Solutions

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institute for Neurological Diseases and Stroke (NINDS)

ABSTRACT
The long-term objective of this research is to understand and develop engineering solutions to the difficulties presented to magnetic resonance imaging (MRI) at high magnetic field strength.

Specific Aim 1: Develop and validate methodology to analyze and quantitate magnetic susceptibility distortion occurring regionally the human body. These solutions will be used to develop distortion-free correction techniques for high-speed functional MRI and distortion-free MRI of human, animal, and cellular anatomy.

Specific Aim 2: Develop and validate models and methodology to analyzing quantitative radio frequency (rf) magnetic field distortion occurring the human head and body of men, women, children, and fetuses. These solutions will be used to evaluate patient safety from absorbed rf energy and to evaluate distortion and limitations of rf field homogeneity and it potential correction.

The results of these studies will aid a wide array of researchers in high speed distortion-free functional MRI, anatomical studies at both low and high field strengths, MR microscopy in animals and intact cells, evaluation of patient safety, and in many cases reclaim techniques which have proven problematic at high field strengths.

STATUS OF RESEARCH AND PARTNERSHIP:
Recent hardware advances have been designed to facilitate unprecedented real-time control of the RF magnetic (B_1) field distribution with multiple variable voltage or current sources for MRI (1-6). This is a valuable development, in part because distinct current distributions in the coil or array used for excitation will likely be required to achieve homogeneous B_1 fields on different planes (7, 8) or in different subjects (9) in human MRI at increasingly high field strengths. These hardware developments also are fueled in part by the expectation that multicoil tailored RF pulses (coordinating simultaneous application of specially-shaped RF and gradient pulses for spatially-selective excitation), such as those described as "transmit SENSE," will be able to achieve volume-selective homogeneous excitation with shorter pulse durations than their single-coil counterparts (2,

10, 11). A few other multi-coil approaches to achieving homogeneous images besides RF shimming and transmit SENSE, requiring differing amounts of foreknowledge of the RF field and varying degrees of pulse sequence manipulation, also have been proposed and discussed (12-14).

While many of these methods can theoretically produce more homogeneous excitations than RF shimming alone, they can require more foreknowledge of the field's distributions (as for designing tailored pulses), longer-duration RF excitations, higher SAR (as for adiabatic and composite pulses) and restrictions on sequence design beyond the excitation portion. Although RF shimming by itself shows much promise, it also inevitably will have limits because, while Maxwell's equations may allow for a perfectly homogeneous RF magnetic (B_1) field distribution over any one plane in an object as large as the human head at frequencies at today's high field MRI's upper limit, acheiving this may require a large number of excitation sources. And achieving perfect homogeneity throughout an entire volume, even with a very large number of excitation coils, may not be possible due to the constraints of Maxwell's equations (7). Here we simulate a large number (16 to 80) of excitation coils using numerical calculations to examine the limits of B_1 field homogeneity that can be achieved by RF shimming alone on various single slices, and over the brain's entire volume, at frequencies from 300 to 600 MHz (Figure 1).

ISSUES

No outstanding issues or difficulties at this time

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PROJECT TITLE: MagScrew TAH Testing thru Pre-Clinical Readiness

PARTNERS:

The Cleveland Clinic Foundation (CCF, Cleveland, OH)
Foster Miller Technologies (FMT, Albany, NY)
Wilson Greatbatch Technologies, Inc. (WGT, Clarence, NY)
Whalen Biomedical, Inc. (WB, Somerville, MA)

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

ABSTRACT

The fundamental goal of the program is to bring 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 functions. (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.

As a consequence of this design and testing effort, surgeons will have another, superior choice among relatively limited TAH alternatives. The "biolized" pump of the Magscrew TAH has pericardial valves combine with biological, protein blood contacting surfaces, and a long track record of extremely rare thrombo-embolic episodes in calves, despite the absence of anti-coagulation. In addition, the Magscrew actuator is the conceptually simplest and most rugged of those available for TAH's, with very few contacting or rubbing surfaces. Mechanical failures have very few possible sources, which clearly increase both reliability and long-term durability. The "fail safe" controller will address the residual pinched wire, corroded solder joint, software hand-up and similar problems that are unavoidable, even with the best fundamental design, and rigorous quality control, in sophisticated, densely packed electronics that are implanted in a hostile environment, and that have caused failures of other, older systems. While the clinical need for TAH's is consistently estimated to be much smaller than that of for VAD's, it is of a size both nationally and internationally to be of commercial significance. In the United States, it may excess $1B per year in potential sales. The TAH market will support several suppliers, if

not as many as now pursuing the VAD market. To those patients who will need a TAH, the potentially very limited supply of alternatives is of literally life and death significance.

STATUS OF RESEARCH AND PARTNERSHIP

The complete implantable system has been integrated and used for calf implants of up to 92 days duration, the planned maximum in-vivo study period. Systems are on bench test, with the high time system over 10,000 hours. External battery packs have been cycled tested to the end-of-life condition. A number of major conclusions have been reached; 1) The MagScrew TAH fully implanted system basically performs as expected; 2) For various reasons the implanted internal battery is not sufficiently reliable for human use and needs to be mechanically and electrically redeveloped; and, 3) Various refinements can be made to the actuator, the transcutaneous energy transmission system, the external mentoring unit and the wiring harness, to make them even safer and more effective in human use. The fail safe features and diagnostic monitoring system are not yet fully implemented, because of insufficient remaining funding. The program is continuing bench endurance and engineering tests and upgrading the engineering specifications and design documentation to fully reflect the maturity of understanding obtained during the past four years of work.

The partnership is evolving in several directions. A new partner with extensive wiring and connector experience has been identified and is beginning to work with the team. Wilson Greatbatch is moving away from developing complete battery packs, as opposed to its core battery cell business, and is working with us to transfer this technology to an appropriate vendor. The business status of Whalen Biomedical is uncertain.

ISSUES

Cost increases require careful consideration of program priorities to maximize the impact of the year 4 funding. A no-cost extension has been requested, and residual funds are being directed to addressing the highest impact areas of the system.

The grant was submitted for renewal twice, and did not receive a fundable score either time. The first review was addressed with significant additional test data and analysis. In the second review, the primary review committee suggestion was that the system should be pushed into clinical trial now, and further engineering development funded after satisfactory clinical experience is obtained. The NHLBI accepts only one re-submit of a $500K+, and has not agreed to a policy modification to allow us to address issues with the second review. The full system can not be ethically placed into a human trial until the team implements and demonstrates a number of now clearly possible safety and effectiveness related design features. A new proposal for a percutaneous system has been submitted and is now pending.

System integration was a major milestone in the program that enables us to launch our in-vivo test program at the CCF. It is clear to us that such a large scale system involving many partners and even more vendors will require a more extensive quality assurance program in the future to facilitate communication among the players, ensure that critical tests are identified performed in a timely manner, and that quality control requirements are promptly defined and imposed on hardware fabrication.

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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 (NIBIB)

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

Materials development and device development work has focused on 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 in development of demonstration devices are silicones or polyacrylate that provide high energy density due mainly 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 mechanical characterization of finished materials and development of analytical models. Models have been developed for circular thin film membrane and annulus geometries. Because of the large strains involved, large displacement 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, processing requirements, and secondary requirements such as electrode application and fixation. We have succeeded in fabricating thin film laminates capable of operating at higher pressures and demonstration of pumping, at modest right heart pressures, in a valved chamber with about 70% of its surface composed of active material.

ISSUES

We have found that allowing graduate students working in different subgroups to interact as colleagues to be a particularly effective means of encouraging flow of knowledge among the labs. This also adds to the students' training experience. Effective partners are motivated chiefly by the desire to work collaboratively on new problems.

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PROJECT TITLE: Dynamic Properties of Bacterial Adhesins

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

ABSTRACT

We have observed that *Escherichia coli* bacteria require mechanical drag force created by shear stress to bind strongly to host cells. In these studies, *E. coli* bacteria bind to host cells via micron-long type 1 fimbria that terminate in an adhesive protein called the FimH adhesin. FimH binds to the terminal mannose residues in the oligosaccharide componets of glycoproteins of host cells. The goal of our BRP has been to determine the molecular mechanism for how mechanical forces enhance FimH-mediated interaction with mannose.

Our goals include: To identify what minimal components of the bacteria and the host cells are necessary for shear/mechanical activation. To determine what structural changes are caused by mechanical force. And, to demonstrate how these structural changes affect adhesive properties such as bond lifetime or bond numbers.

To address these questions, our partnership applies a mixture of nanotechnology, protein biology and microbiology tools. These tools include purification, functional analysis and crystalization of components of the adhesive machinery, site-directed mutation of the FimH adhesin, steered molecular dynamics simulations of the effect of force on the structure of FimH, experimental FRET studies of the effect of force on protein structure, flow chamber experiments of bacterial behavior in flow, and single molecule force studies.

This work is of interest on two levels. First, we need to understand how bacteria adhere to tissue and biomaterial surfaces in order to prevent this adhesion, since adhesion is the first step to infection and biofilm formation. Second, we can learn how to design small mechanically sensitive nanotools that respond to mechanical forces.

STATUS OF RESEARCH AND PARTNERSHIP

In the past year we have had tremendous success in a range of sub-projects supported by this BRP. This has resulted in the acceptance of seven papers and the submission of another four since this time last year, in addition to a number of exciting preliminary results. Most of the results have involved tight collaboration between three or more research groups. We briefly describe our most important discoveries since the start of this grant period:

We have shown that shear-enhanced *E. coli* adhesion is due to the FimH-mannose interaction by reproducing it in cell-free assays and by eliminating possibilities such as transport effects. We have also elucidated a large part of the mechanism of shear activation. FimH undergoes a force-induced extension

in a regulatory region of the protein that is allosterically linked to the mannose-binding site and causes a switch from a short-lived to a long-lived state of the bond. This means that FimH is a “catch bond” that is longer-lived under higher forces. However, the details of the allosteric mechanism remains to be determined – that is, we do not know how the structural changes in the regulatory region change the binding site.

We have also made a number of discoveries that demonstrate the importance of shear-enhanced adhesion. We have shown that shear-enhanced adhesion can be observed even when bacteria bind well at low shear, when one observes the manner in which bacteria bind – that is, whether they roll or are stationary - and not just the number bound. Using this method, we have shown that shear-enhanced bacterial adhesion is more common than previously thought, as it is observed for other receptor ligand pairs as well. We have also identified several advantages to the bacteria for shear enhanced adhesion. It prevents detachment by soluble inhibitors, and allows more stable and rapid surface colonization during the early stages of biofilms than does conventional modes of adhesion. Finally, we have discovered that the fimbriae on which FimH is presented undergo a force-induced uncoiling and recoiling behavior that optimizes the force on the catch bonds, indicating that *E. coli* have evolved multiple mechanisms for shear enhanced adhesion.

The importance of this work extends beyond the field of bacterial adhesion. Our mathematical models and structural theories have benefited those trying to understand catch bonds and shear enhanced adhesion in systems from blood cells to motor proteins. Our demonstration of the role of mechanical force and of ligand-induced changes in binding in allosteric regulation present novel concepts in protein structure and chemistry. Finally, we have demonstrated applications of this work such as development of a microfluidic shear stress sensor.

ISSUES

The partnership has adjusted well to the addition of a new member, Wendy Thomas, who is an assistant professor of Bioengineering at the University of Washington, and to the move of Viola Vogel from the University of Washington in Seattle to ETH-Zurich, Switzerland. We have had twice yearly meetings alternating between Zurich and Seattle and additional extended stays by individuals at each institution to perform experiments and exchange expertise and data with other groups. We talk regularly on the phone overseas and email almost daily. This has preserved the close collaboration that has made this project successful.

Our major issue at this time is securing funding to continue this work, as we have just submitted our competing renewal application.

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PROJECT TITLE: Regenerative Scaffold Technologies for CNS and Diabetes

PARTNERS' NAMES AND AFFILIATIONS:

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William Lowe (Endocrinology)

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

ABSTRACT

This Bioengineering Research Partnership (BRP) focuses on two specific challenges of great clinical importance, regeneration of the central nervous system (CNS) and cell replacement therapies for diabetic patients. The target of the team is to develop multiple scaffold technologies and use CNS regeneration and pancreatic tissue replacement as a testing ground. 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 cross-linking of soluble bioactive peptides.

STATUS OF RESEARCH AND PARTNERSHIP

Significant progress has been made toward developing regenerative scaffolds for the central nervous system (CNS) and for the transplantation of pancreatic islets lost to diabetes. The Stupp laboratory has synthesized bioactive, self-assembling peptide amphiphile (PA) nanofiber gels, including a neurobioactive system with the peptide epitope IKVAV. Gelation kinetics were modified, while preserving bioactivity, by systematic changes to the molecule's backbone peptide sequence. This was a crucial enabler of translational in vivo studies, allowing sufficient working time for complete injection into the spinal cord prior to gelation. The Stupp and Kessler labs have shown that culturing progenitor cells in these self-assembling gels enhanced neuronal lineage commitment and inhibited astrocytic differentiation. To test the hypothesis that the amphiphile acted on the progenitor cells via integrin signaling, the Kessler laboratory demonstrated that treatment with an inhibitor of ILK significantly reduced the effects of the PA on lineage commitment. Further, this integrin signaling specifically blocked the effects of BMP signaling without altering effects of signaling by LIF.

We have also studied the effect of these self-assembling IKVAV PAs on recovery from spinal cord injury in a rat model. Injection of the self-assembling scaffold significantly reduced 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, we found significant functional improvement, as measured by Basso, Beattie, and Bresnahan (BBB) scoring and by an incline plane test. Histopathological evidence showed that the IKVAV PA promotes regeneration of motor axons following spinal cord injury. We examined tracing for ascending proprioceptive fibers and descending corticospinal fibers. At 2 weeks post-injury only a few axon fibers penetrated into the lesion. However, by 11 weeks 40% of the axons in the IKVAV PA scaffold-injected group penetrated through the lesion into the caudal spinal cord. By contrast, no axons were ever seen crossing through the lesion in the vehicle-injected control animals.

The Shea laboratory employed a salt-leaching technique to create microporous scaffolds from a mixture of poly(lactic-co-glycolic acid) (PLG) microparticles, resulting in an interconnected, three-dimensional, porous bridge after gas foaming and particulate leaching. Multiple small channels within the bridge have been proposed as possible reconstructed pathways for growing axons, and also can serve to deliver cell stimulatory factors (protein, DNA). Such bridges were implanted by the Kessler laboratory into a rat hemi-section spinal cord model and found to retain their position and tissue apposition at the implantation site up to 4 weeks. PLG bridges are being developed that release proteins, such as NT-3, that target the different barriers to spinal cord regeneration. Initial studies with these bridges have shown enhanced axonal extension into the channels. Furthermore, we have investigated the delivery of chondroitinase ABC, which is aimed at reducing the inhibitory chondroitin sulfate proteoglycans that can accumulate within the glial scar, with similarly positive preliminary results.

As a compliment to short, self-assembling synthetic peptides, the Barron laboratory has produced high-molar-mass recombinant proteins as building blocks for comb-like macromolecular architectures with reactive lysine residues for attachment of different functionalities. Side-chains produced include tissue transglutaminase peptide substrates for enzymatic cross-linking and α-helical segments that can form dimeric coiled-coils and enhance network structure. Initial cytotoxicity tests demonstrated the protein polymer hydrogels act as a nontoxic substrate for cell adherence. Rheological studies show that the cross-linked system forms viscoelastic gels with a storage modulus (G') of 6 kPa. Simple post-translational modifications were used to incorporate functional moieties into the resulting hydrogels, including gadolinium chelators as contrast agents for magnetic resonance imaging (MRI) and peptide-based nuclear localization signals (NLSs) for non-viral gene delivery.

The Kaufman laboratory continues to develop mouse models to examine the effect of pancreatic islet transplantation on streptozotocin-induced diabetes, including two new implantation sites—the epididymal fat pad and the subserosal site of the small intestine. These sites complement previously established intraperitoneal (omentum) and subcutaneous implantation sites, and offer advantages because the islets are easier to visualize post-transplant. Islet transplantation has proven effective at treating type 1 diabetes; however, during islet isolation, enzymatic digestion disrupts key contacts between the cell and the extracellular matrix, leading to decreased islet mass and function. To recreate the islet niche using synthetic biomaterials, low density, enzymatically cross-linked peptides could serve as a cell delivery matrix. The Messersmith laboratory has cross-linked biotinylated phenylalanine-lysine-glycine (FKG) peptides with the basement membrane of murine islets. Biotin surface functionalization of the islets provides a general platform whereby peptides, nucleic acids, and small molecular weight pharmaceuticals can be delivered in a controlled manner. Enzymatically cross-linked poly(ethylene glycol) networks are being used by Messersmith and Kaufman to optimize islet transplantation via in situ gelation and encapsulation of islets within the epididymal fat pad. Successful engraftment of the transplanted cells within the host tissue was demonstrated.

The Shea laboratory has fabricated microporous PLG scaffolds, similar to the spinal cord bridges described above, capable of controlled DNA and protein release to promote islet engraftment. Preliminary studies have used micro-CT imaging to characterize vascular in-growth into the scaffold, demonstrating that increasing the local concentration of vascular endothelial growth factor (VEGF) leads to a dramatic increase in blood vessel density at 3 weeks, relative to control scaffolds. Studies in collaboration with the Lowe laboratory established that when diabetic mice were transplanted with either 280, 175 or 125 islets on a PLG microporous scaffold, the mean time to the resolution of euglycemia was 1.0, 3.9, and 20.0 days following transplantation, respectively. The islets maintained function for at least 180 days and histology demonstrated re-established of a functional vasculature. Importantly, islet function was significantly better than identical transplantations without the PLG scaffold, and bioluminescence imaging demonstrated that islets transplanted on the scaffold remained localized to the peritoneum, whereas those transplanted onto intraperitoneal fat became dispersed throughout the abdomen. The ECM composition on the scaffolds has also been investigated for its ability to enhance islet engraftment. Fibronectin, collagen IV, and laminin were investigated by adsorbing to PLG scaffolds prior to islet seeding and scaffold implantation. Collagen IV coating of the scaffolds significantly enhanced islet engraftment and function relative to controls, and incorporation of collagen IV coatings with the protein and DNA delivery is now being explored. DNA delivery from PLG scaffolds is also being explored as an alternative to protein delivery. The Shea laboratory examined the role of molecular weight, pore size, and scaffold geometry on the duration of expression or the amount of expression when used to deliver DNA in vivo. These scaffolds do result in transgene expression, both in the subcutaneous space and in the intra-abdominal fat, which are potential sites for islet transplantation.

ISSUES

None of note. Research and administrative efforts are progressing smoothly.

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PROJECT TITLE: Complex Nanocomposites for Bone Regeneration

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GRANTING NIH INSTITUTE/CENTER (IC): National Institute of Dental and Craniofacial Research (NIDCR)

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 are being 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 third year of the project has been very productive with significant progress having been made on most of the specific aims. During the third year we focused on three areas: (1) new techniques for the preparation of porous inorganic and hybrid organic-inorganic scaffolds; (2) fabrication of dense hybrid materials, and (3) analysis of wetting and adhesion at the organic-inorganic interface.

1. Fabrication of porous scaffolds. Two techniques have been developed for the fabrication of porous scaffolds: robotic assisted deposition and freeze casting. Robotic assisted deposition has been used to fabricate ceramic and hybrid organic-inorganic scaffolds with computer designed architectures. The development is based on the formulation of inks with suitable viscoelastic properties for the fabrication of complex 3-D structures. The ink should flow through a nozzle and subsequently settle very fast, bonding to the previous layer so that the part maintains its shape while printing. The pure ceramic scaffolds are made of hydroxyapatite (HA) or β-tricalcium phosphate (β-TCP) and have compressive strengths of the order of 10-25 MPa, slightly higher than cancellous bone. In the hybrid materials, HA or bioactive glasses are mixed with either poly-l-lactic acid (PLA) or polycraprolactone (PCL). Consolidation of the hybrid scaffolds is achieved by matching the printing speeds to the drying kinetics of the ink. The hybrid scaffolds can contain up to 70 wt% of an inorganic phase homogeneously distributed in the polymer and

do not fail in a brittle manner during compression tests showing an elasto-plastic response with large plastic "yielding". Their mechanical response is clearly anisotropic and can be easily adjusted by controlling the composition. The addition of an inorganic phase significantly increases the stiffness, and the Young's modulus of scaffolds containing PCL is typically between one half and one third lower than those of the scaffolds prepared with PLA.

Freeze casting is based in the development of a novel experimental setup for the controlled directional freezing of ceramic slurries. During freezing the ceramic particles concentrate in the space between the ice crystals. Afterwards, the ice is sublimated by freeze drying, such that a layered ceramic scaffold whose microstructure is a negative replica of the ice is produced. By increasing the freezing rate a finer microstructure is obtained, without affecting the long range order of the entire structure. In this way, we have prepared lamellar microstructures with layer thickness varying over two decades, from 1 μm (almost the same as nacre, typically ~0.5 μm) to 200 μm. By controlling the freezing kinetics and the patterns of the cold finger, it is also possible to build mesostructural features and gradients that could be used to optimize the mechanical response of the materials. The porous scaffolds obtained by this process exhibit striking similarities with the meso- and micro- structure of the inorganic component of nacre. The inorganic layers are parallel to each other and very homogeneous throughout the entire sample. Particles trapped in between the ice dendrites led to a surface roughness of the walls, just as in nacre. Finally, some dendrites span the channels between the lamellae, mimicking the tiny inorganic bridges linking the inorganic platelets of nacre, which are believed to increase the fracture resistance. These highly porous lamellar scaffolds can be up to four times stronger in compression than conventional porous hydroxyapatite. They exhibit well-defined pore connectivity along with directional and completely open porosity. Hence, most of the current shortcomings (low strength, random organization, multiple pore size, uncontrolled pore connectivity) that plague such bone substitutes are solved by this innovative approach.

2. Preparation and characterization of dense hybrid materials. A novel technique for the fabrication of dense PLA/HA composites has been developed. The process is based in the fabrication of PLA/HA microspheres using an emulsion-solvent evaporation method. The microspheres are used as building blocks to produce dense, homogeneous composites by hot pressing. This route allows us to prepare composites with HA contents as high as 60 wt%. The stiffness of the composites matches well that of cortical bone (Young Modulus, 12 GPa) and the strength and toughness are higher (bending strength, 120 MPa; and Strain Energy Release Rate, 200 J/m^2). While the elastic modulus was relatively unaffected by *in vitro* degradation in Hanks' Balanced Salt Solution, both the strength and the fracture toughness decrease 30 to 40% due to the degradability of the polymer phase and the observed decrease in adhesion at the organic/inorganic interface. However, they remain above that of cortical bone and can be adequate for many load-bearing applications.

4. Wetting and Adhesion at the organic-inorganic interface. Sessile drop experiments combined with surface tension measurements have been used to measure the thermodynamic work of adhesion between diverse biopolymers and hydroxyapatite. The works of adhesion range between 55 and 62 mN/m. The two forms of polylactide (l- and d-) had nearly the same work of adhesion, which is expected. The racemic form of polylactide (PDL) had a slightly higher value while the addition of polyglycolide as a copolymer to PDL was found to lower the work of adhesion. Indentation and four point bending tests were used to evaluate the mechanical strength of the polymer/ceramic interface. The interfacial strengths are very similar (between 15 and 22 MPa in 4-point bending) and correlate well with the works of adhesion. However, after exposure to a humid environment at 37°C the strengths decrease significantly. It is clear that there is a strong effect of humidity on the adhesion at the polymer-ceramic interface that will have strong effects on the in vivo performance of hybrid organic/inorganic composites.

ISSUES

None

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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 (NHLBI)

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, is used. A tissue mechanical theory is 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 is used to define what alignment-dependent mechanical properties of our "valve-equivalent" (VE) are desired for proper valve function and to simulate what the VE function will be. Various experimental strategies are implemented to manipulate these properties during incubation. Measurements of these properties are used to develop the needed microstructural constitutive model of the tissue resulting from the cell-remodeled gel. High-speed ultrasonic imaging of leaflet motion and 3D particle image velicometry are used to validate the model as well as visualize valve function. Novel adult stem cells are being assessed as a source of surrogate interstitial leaflet cells for VE fabrication. An animal study is ultimately planned.

STATUS OF RESEARCH AND PARTNERSHIP

Valve-equivalent Fabrication.

Cell confluence, passage number, plating density, and use of conditioned media all were found to influence the amount of collagen production by human dermal fibroblasts (HDF) and porcine valve interstitial cells (PVIC) in adherent fibrin gel discs. The optimal conditions for maximal UTS were use of fibrin gel at low initial concentration, high-passage confluent cells, and conditioned medium. Preliminary studies were completed using tubular constructs to determine the effect of cyclic distention on fibrin remodeling. Results using PVIC indicate that cyclic distension increases tensile mechanical properties over static controls at 3 and 5 wk time points. These data will be used to prescribe initial cyclic strain protocols for a VE bioreactor currently being tested.

VEs fabricated using both HDF and PVIC possessed circumferential alignment in the root and leaflets. After ~4 wk of static culture on the mold, VE tissue ranged from 10-25% collagen. Uniaxial tensile testing of HDF-based VE leaflets in the circumferential direction showed an average tensile strength and modulus of 0.4 and 1.4 MPa vs.1.1 and 11.0 MPa for similarly tested native leaflets. VEs were capable of sustaining over 250 mmHg back-pressure before failure. We successfully embedded Dacron rings into the ends of the VE root to provide a suturing site, without negatively affecting the cell-mediated fibrin contraction that confers alignment. A large volume of histological, compositional, uniaxial mechanical and planer biaxial mechanical data have been collected for both VE tissue and porcine valve tissue and are being analyzed currently.

Particle Image Velocimetry

Experiments are in progress to characterize flow through rigid and deformable diaphragms viewed as aortic valve analogues. The diaphragms are located in a pipe flow downstream of a long development section.

Inflow conditions examined thus far include fully developed laminar and turbulent velocity profiles that are steady in the mean. PIV is used to document the initial 'inflow' conditions as well as instantaneous flow fields downstream of each valve. At the same time, the valve deformation is captured on the PIV images. The deformation and velocity data will be used in combination to test the accuracy of the fluid/structure interaction numerical code developed as part of this project. Additional experiments have been performed in order to optimize the design of a pulsating flow loop containing resistance and capacitance elements necessary to mimic physiological pressure drop and flow rate signatures through valves.

Ultrasound Imaging

We have demonstrated the feasibility of imaging the VE through a latex tube. Two-dimensional displacement tracking based on real-time (100 fps) imaging has also been performed with our small parts probe to enable measurement of strains in the bioreactor.

Assembly of a 3-stage servo system for 3D imaging is complete. Excellent imaging results of the HVE are now routinely obtained within minutes vs. hours (with a 3-stage stepper motor). The image acquisition system employs coded-excitation (arbitrary waveforms with frequency components up to 80 MHz). Improvements in SNR up to 12 dB above conventional pulse-echo systems are possible with 4 s chirp waveform duration. Our system is now capable of real-time imaging at 10 frames per second (400 line images with lateral spacing of 50 μm). A Vertix IIPro FPGA (field programmable gate array) board is used for data collection and real time processing.

We are simulating array transducers in the 20 – 40 MHz range in preparation to design and fabrication of a prototype array to be ordered from the NIH Resource Center for Medical Ultrasound Transducers. We have tested PVDF 16-element arrays on printed circuit boards and established that they are still capable of generating piezoelectric response. Despite their lower sensitivity compared to PZT transducers, the high bandwidth of PVDF transducers and the use of coded excitation may allow us to regain much of this sensitivity in practice. The feasibility of high frame rate array imaging at high frequencies (20 – 40 MHz) will be established within the current funding period.

Computational Valve Mechanics

Using a 3-D code for regular geometries based on our anisotropic biphasic theory of tissue-equivalent mechanics, we studied development of alignment in an idealized leaflet forming in the VE mold. It revealed how the length of the flap (the unconstrained portion of the initially-formed leaflet) affects the ensuing alignment in the peripherally-anchored portion of the leaflet. Difficulties in treating irregular contact surfaces with our code were overcome by introducing a thin lubricating layer of low-viscosity fluid between the VE and the mold surface. This layer both represents physical reality better and provides better computational performance. We have completed tests on this new method and are now applying it to simulate the early-stage compaction of the valve-equivalent.

Our 2-D overlapping-grid scheme for the valve leaflet has been completed. The model uses three grids: solid (valve leaflet), near-valve fluid (modeled using the arbitrary Lagrangian-Eulerian, or ALE, deforming mesh approach), and free fluid (modeled using a fixed mesh). The solid and near-valve fluid meshes are conforming, and the two of them float over the fixed mesh. Communication between the two fluid meshes is accomplished by a distributed Lagrange multiplier. We are completing an initial 2-D transient study, demonstrating that we can model valve motion in moderately fast flows (Re ~ 500) accurately and efficiently. We are currently extending the model to three dimensions, which will allow simulation of the steady flow pressure drop measurement, an essential test of valve performance. Upon completion of that study, we will extend the code to model leaflet-leaflet contact, permitting simulation of the full range of valve motion.

Significance

We have demonstrated the ability to fabricate a fibrin-based VE, to perform 3D PIV, to use a wrapped-embedding approach for simulating leaflet motion, and to image VE leaflets and displacements 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 (NIBIB)

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?

STATUS OF RESEARCH AND PARTNERSHIP

Within the past 5 years, there has been an explosion of visual prosthesis research, within the U.S. and worldwide. Most of these efforts target the retina as the location of an artificial interface between a camera-based imaging system and the human visual system. It is anticipated that, unlike the cochlear implant, hundreds or thousands of parallel stimulation channels will be required for a perceptually useful visual prosthesis. The development of reliable implantable hardware and establishing strategies for neural coding remain unrealized. IIT leads a multidisciplinary team for the development of an intracortical visual prosthesis using large numbers of intracortical micro-electrodes that penetrate the visual cortex. The overall objectives of our multi-institutional team-based project are to advance the technology

sufficiently to provide a reasonable expectation of reliability and safety for implantable hardware, to develop an animal model to perform crucial psychophysical and electrical stimulation studies, and to consider key ethical issues, so that a multi-model decision process about proceeding to a human volunteer can be defined and implemented.

Although considerable research had been historically supported by the NIH for the development of safe intra-cortical microelectrodes, our in-vitro and in-vivo testing of over 400 electrodes in bench electrochemical analysis, and 3 different animal models, confirm that the in-vivo charge injection capability of these electrodes is significantly less than had been assumed. Our working hypothesis is that limitations in counter-ion availability, within the brain, impede the charge injection process. We have defined a new paradigm for defining the safe charge injection limits based upon potential excursions of the electrodes during stimulation, rather than apriori definitions of charge injection capacity. Using this new electrode qualification process, we are presently assessing a modified electrode design that would be suitable for human implantation. These results transcend our visual prosthesis project and affect all neural prosthesis research.

Although the prospect of human implantation for an intracortical visual prosthesis has often seemed uncertain, we have made significant progress in understanding how such a system might be configured. We have developed self-contained 16-channel wireless modules that use new methods of electrode array assembly, as well as our design for an application-specific-integrated-circuit that drives the microelectrodes using a rule-based protection method. In a first volunteer, we plan to implant 3-4 of these modules in order to establish the stability of the artificial neural interface. Once the stability of the neural interface has been demonstrated, it seems feasible to perform an additional surgical procedure to provide the volunteer with additional modules, up to approximately 1000 electrodes, that would cover the surface of the occipital pole.

After some unexpected detours in our original project plan, we are now confident that a first-generation intracortical visual prosthesis can be safely implemented in a human volunteer.

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 characterization of electrode charge capacity, in-vivo, and the qualification of the wireless module design. The timetable for a human implantation.

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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 (NHLBI)

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 third year of this BRP project, research is progressing as planned. Analytical investigation: Using Moffatt corner eddy flow as a model of alveolar flow, we have been investigating how the interaction of wall motion with recirculation affects kinematic irreversibility. This year, we investigated the qualitative and quantitative contributions of the parameters toward the amount of chaos exhibited by the corner flow. In particular, we examined whether the extent of chaotic motion is affected by the degree

of phase shift between alveolar wall oscillation and the periodic ductal flow. We showed that for weakly varying unidirectional flow, the self-similar structure hardly changes from when the unidirectional flow is constant (the moving corner model). For strongly varying bidirectional flow, the self-similar structure is enlarged. However, when both the alternating and unidirectional contributions of the far-field flow are sufficiently large and similar in size, chaotic transport is enhanced. Additionally, we found that incorporating a phase shift into the alternating flow reorients the self-similar structure. It can also control the extent of chaos. Computational investigation: Continuing our previous efforts, we are currently finalizing the development of a 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 continue developing a nose-only inhalation apparatus for the developing rat (7, 14, 21 day old or adult WKY/Kyo rats; radio-labelled ^{192}Ir 20 nm and 80 nm aerosols). Principal findings are, i) The total deposition peaks in 21 day old rats (68% or 63% with 20nm or 80nm, respectively), consistent with the idea that the extent of chaotic mixing, and consequently the enhancement of acinar deposition, would be the highest in 21 day old rats where the acinus has already become largely alveolated but alveoli are still small in size, ii) Airway vs. alveolar deposition fractions are distinguished by the fraction which had been cleared from the respiratory tract within 24 hours vs. the fraction which was still retained in the lungs after 24 hours. The alveolar deposition is always higher than the airway deposition for both 20nm and 80nm particles; both airway/alveolar deposition peaks in the 21 day old, supporting our theoretical prediction. (2) This year, we have redesigned a PC-controlled high precision lung function unit, with which deposition can be evaluated from a very large number of breaths (almost 1000 breaths per animal). To test this new unit, experiments were performed in 35-day old male WKY-rats. A breath-by-breath analysis of intrapulmonary particle deposition was monitored, as well as the corresponding breathing parameters, including: respiratory rate (f), inspiratory/expiratory tidal volume (Vt and Vtex, respectively), minute ventilation (MV), inspiratory time (Ti), expiratory time (Te), relative duration of inspiration (Ti_TT), peak inspiratory/expiratory flow rate (PIF and PEF, respectively), and mean inspiratory/expiratory flow rate (MIF and MEF). Generally, there is a considerable amount of breathing pattern variability. Approximately 900 breaths were analysed for one of the rats, and an empirical equation of intrapulmonary particle deposition was derived. The initial results (tested in three rats with 2 μm particles) show that (i) for a given tidal volume, deposition decreases with increasing respiratory rate and (ii) deposition increases with increasing tidal volume at a fixed respiratory rate.

BRP partnership meetings were held several times. The Harvard-GSF partnership meetings were held on May 23, 2006 in San Diego where the PI and the key personnel of the GSF site (Drs. Kreyling, Schulz and Semmler) exchanged the updated information on the research progress of each site. In addition, Dr. Kreyling and Dr. Semmler-Behnke visited at the Harvard site (Boston) on April 23 and May 25-26, 2006, respectively, where the PI and the investigators discussed the progress in detail. The Harvard-Kragujevac Partnership meeting was held on June 27-30 at the Kragujevac (Serbia) where the PI and the key personnel of the Kragujevac site exchanged the updated information on the research progress of each site. The Harvard-Surrey-GSF Partnership meeting will be held on August 3, 2006 in Munich where Dr. Laine-Pearson will report on the progress of the Surrey site to the PI and the GSF group members.

ISSUES

None.

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PROJECT TITLE: cGMP Recombinant FIX for IV and Oral Hemophilia B Therapy

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

ABSTRACT

The hemophilias are congenital disorders characterized by frequent bleeding episodes, especially into the joints and muscles, that cause severe arthritis and crippling. Untreated patients rarely survive past adolescence. The most common hemophilias are caused by congenital deficiency in the clotting proteins Factor VIII (Type A) or Factor IX (Type B), which occur in about 1:5,000 and 1:23,000 male children, respectively. These patients are currently treated with replacement therapy using intravenous administration of purified Factor VIII or IX produced by purification from donor plasma or in cell culture bioreactors.

The objective of this research is the rapid development of a safe, efficacious, abundant, and inexpensive source of recombinant human Factor IX (FIX) that is ready to be evaluated in clinical trials. Our approach capitalizes on two important features: (1) existing genetically engineered transgenic pigs that synthesize 40 IU/ml/hr or more of transgenic Factor IX in milk relative to 0.08 IU/ml/hr in the currently available Chinese Hamster Ovary cell bioreactor; and (2) favorable pharmacokinetic properties of the transgenic Factor IX in preliminary studies in a hemophilia B mouse model. This unique combination of abundance and quality is the basis of the need for definitive bioengineering research and development that will enable first an intravenous therapy, and subsequently, a non intravenous therapy that can be expected to revolutionize treatment of hemophilia B worldwide. The partnership emphasizes the evaluation of the transgenic FIX material in hemophilic B animal models where this material has been made by good laboratory practices (GLP) that are amenable to current good manufacturing practices (cGMP). Thus, the reliability of the pre-clinical data is better ensured as is the potential for more efficient scale-up of candidate materials for eventual clinical study.

STATUS OF RESEARCH AND PARTNERSHIP

Our funding period began on September 6, 2005 and we have completed the following first year goals:

1. Installation of inter-laboratory handling (cradle to grave) guidelines and documentation as required by the USFDA for transgenic milk and its processed intermediates of the transgenic FIX.
2. Establishment of biochemical characterization methods needed for future GLP and then cGMP quality material that follow USFDA precedent for previously approved recombinant FIX products.
3. Initiation of routine screening of milk from different transgenic pig lineages and establishment of milk pooling guidelines by identifying those milks containing the greatest abundance of FIX populations that have acceptably high specific procoagulant activities.
4. Advancement of a previous NIH-NAID investigation that helps to initially define some of the salient immunogenic/tolerigenic properties of the transgenic FIX in oral delivery mode in the hemophilia B mouse model using raw transgenic milk containing 4 g/l of FIX.
5. Development of at least five candidate hybridoma lineages that produce monoclonal antibodies that have metal-dependent binding of FIX that will provide gentle elution conditions for immunoaffinity purification of FIX. The safest, most efficacious and economical FIX product available today is immunoaffinity purified. Immunoaffinity purification should provide a robust, almost universal barrier of 3 to 6 orders to pathogen contamination as well as a reproducible selection for desirable populations of acceptably, post-translationally modified FIX that provide a long circulation residence time.
6. Determination that a sequence of Heparin affinity, anion exchange, and immunoaffinity chromatographies will provide a robust, high yield and cost effective purification process for producing high specific activity FIX from transgenic pig milk.
7. Initiation of FIX formulation technology for non-intravenous administration of FIX using candidate fractions consisting of 98%+ pure material suitable for normal and hemophilic B mouse model evaluation.

The coming year will utilize these tools and the partnership to efficiently select (based upon structure and *in vitro* function) the most promising of FIX populations for pharmacokinetic studies in hemophilic B mice. Thereafter in years three through five, candidate FIX populations will be produced and formulated into intravenous and non-intravenous therapies under GLP (cGMP amenable) processes that will be studied in hemophilic B dog models.

ISSUES

The partnership has responded well to in-person meetings every two to three months. This has been essential to the decision process for coordinating numbers of animals available for pharmacokinetic studies with the amounts of purified FIX and then formulation of those materials for delivery. These meetings have provided essential data review and roundtable discussion so as to be able to choose with agility the evolving, iterative path for identifying those abundant FIX populations with the most desirable and promising pharmacokinetic properties and biochemical structure.

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PROJECT TITLE: Long Wavelength Quantum Dot-based Probes for Cell Tracking and Tissue Engineering

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

ABSTRACT

We are developing new technologies to employ quantum dots (QDs) in biological imaging in vitro and in vivo. Quantum dot technology has been developed specifically for (1) cell identification and tracking of cells in tissues, (2) tracking cell proliferation through multiple generations in tissues (3) vascular imaging, and (4) deep 3-D imaging of QD-labeled polymer scaffold structures relative to the QD-labeled cells in engineered tissue models. Quantum Dot Corporation (QDC—now a part of Invitrogen) prepared near-infrared and far-red emitting and surface modified QDs that have properties suitable for deep imaging of cells and structures in tissues. The Molecular Biosensor and Imaging Center at Carnegie Mellon University has developed methods to derivatize QDs for existing and new applications in cell biology. Using QDs provided by QDC, the MBIC prepared new coatings and conjugates to label cells by a variety of means, then tested the labeled cells for fluorescent brightness, stability of labeling, and cell survival and function. As newer QDs become available, they will be similarly tested and compared with existing QDs and existing organic fluorescent probes, e.g., Alexa dyes and cyanine dyes.

To obtain feedback for the development program we have maintained a collaboration with Phil Campbell and Lee Weiss at Carnegie Mellon University. We examined the utility of the developing QD technology for studying cell location, movement and proliferation in the 3-D structures of engineered bone tissue. This is a particularly challenging and relevant system that requires QD technology to extend cell tracking to denser and more highly scattering tissue matrices, including hydroxyapatite-containing artificial bone matrices. We have demonstrated the ability of our vascular probes and our cell tracking reagents to perform time-resolved multicolor imaging at millimeter depth in many natural and artificial tissues, in vitro and in vivo. The technologies we have developed appear to be broadly applicable in many biological and medical applications.

STATUS OF RESEARCH AND PARTNERSHIP

Our partnership began in 2001, and the research project was funded in April of 2002. QDC has been an effective partner in both producing QDs and in devising new surface coatings for use in

biology. During the past four years we have used several different surface coatings to stabilize QDs for use in aqueous solution. By modifying these surfaces, we have:

(1) Greatly increased spontaneous cellular uptake of QDs in vitro (Lagerholm *et al.* Nano Let. 4, 2019-2022; this method has been commercialized by QDC.) Recently, we have greatly reduced the granularity of the cell labeling, resulting in cells that are more homogeneously labeled. We expect this to be a more appropriate cell labeling method for tracing and measuring cell generation after initial labeling.

(2) Labeled engineered tissue matrices and invading cells; we were able to follow cell migration to printed areas of growth factors in engineered matrices. We mastered injection into the developing chick vascular system, then implanted engineered matrices onto the chick chorioallantoic membrane, and followed cell migration, biological effects of immobilized growth factor concentration gradients, and neo-angiogenesis in real-time and with improved sensitivity. Details of vascularization and neovascularization were made visible with unprecedented sensitivity and clarity. Nascent and very small capillaries were clearly displayed without substantial leakage of the QDs from the vessels. Cyanine dyes were easily usable with QDs.

Use of multicolor fluorescence enabled simultaneous investigation of vasculature and matrix invasion. QD-tagged fibrin clots allowed sensitive display of early matrix remodeling. We are extending these studies by differential labeling of other matrix components and invading cells.

Optimized fixation methods were developed to use fluorescence and electron microscopy on the same sections. Glutaraldehyde followed by light osmium fixation gave good results; formaldehyde and ethanol were unsatisfactory. Standard TEM embedding resins (EPON and LRWhite) preserved fluorescence.

In addition to our 3-D grating image and tomographic techniques, we are collaborating with the Pittsburgh Supercomputing Center to create high-resolution 3-D images from sections examined by both fluorescence and TEM for comparison with our 3-D images of living tissue and also to gain EM-level resolution of cell-matrix interactions. The PSC is experienced in aligning and warping large data sets for 3-D rendering (*C. elegans* cooperative rendering project, Visible Human project.) We expect that these methods can be used with engineered tissues in living animals.

Our results on cellular migration in engineered 2-dimensional matrices have been submitted for publication. Our results on 3-dimensional matrices as they relate to neo-angiogenesis are currently in preparation.

(3) Increased circulating lifetime *in vivo* from minutes to several hours; reasonably long circulating lifetimes are required for targeting *in vivo* (Ballou *et al.*, Bioconjugate Chemistry 15 (1), 79-86.) An extension of this chemistry has been commercialized by QDC.

(4) Coupled QDs to biological materials for labeling and targeting. At least some of our polymer-coated QDs retain their fluorescence *in vivo* for at least two years.

(5) Extended emission wavelengths of our QDs to the near infrared (850nm emission), with good stability and quantum yield. These infrared QDs allow deeper imaging *in vivo* and in tissue matrices.

(6) Developed surfaces for minimal interaction with blood components; these led to practical conjugatable derivatives having high performance in flow cytometry. This work is currently in press at Nature Medicine (Chattopadhyay, et al., Nature Medicine, 2006)

ISSUES

After the acquisition, we have continued to rely on Invitrogen as a supplier of QDs, but we have shifted to preparing new surfaces and materials in-house.

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PROJECT TITLE: Cardiopulmonary Organ Engineering

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

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 we have developed our rat surgical model and proceeded to investigate our best scaffolds to date (developed by the Matrix Synthesis Core) as full thickness

replacements 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. We then moved to placing the patch directly over left ventricular infarcts in the rat model and demonstrated improved cardiac function with the scaffold patch. Here again, we have utilized ePTFE as a control material in addition to infarct controls. This work is now being scaled for large animal studies. 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. Porcine and human MDSCs have been isolated and are being characterized.

Related to the construction of a tissue engineered vascular graft (TEVG) we have implanted tubular biodegradable PEUU polymers fabricated by the Matrix Synthesis Core and seeded with mouse muscle-derived stem cells (mMDSCs) provided by the Precursor Cell Isolation Core in the rat model. The constructs have an inner diameter of 1.3 mm and a wall thickness of 200μm. 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 explored the utility of the device as a means for fast and efficient luminal surface seeding.

In our matrix synthesis activities, we have synthesized a growing family of biodegradable poly(urethane)ureas that act as thermoplastic, biodegradable elastomers. We have developed synthetic polymers that incorporate specific peptide sequences for designed enzymatic degradation. 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. Anisotropic scaffolds both with and without cells have been created that closely approximate cardiovascular tissue 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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PROJECTS TITLE: High Resolution SPECT/CT Imaging of Systemic AA-Amyloidosis in Mice

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GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorder and Stroke (NINDS) & National Institute of Biomedical Imaging and Bioengineering (NIBIB)

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: By the end of yr. 3 a dual-head microSPECT/CT hybrid platform is now commercially available through Siemens Medical Solutions Molecular Imaging (Knoxville, TN) and a next generation machine based upon the ORNL architecture is in production. In the last 12 months, during our transition to UT Medical Center, we have evaluated a number of methods to label the amyloidophilic tracer SAP with nuclides other than I-125. To this end, we have had considerable success in certain areas but still need to generate other chelators for positron-emitting isotopes. We have also spent time evaluating new sources of human SAP which has required new methods to isolate and purify the material. We have successfully generated SAP in house and are presently comparing the in vivo and physical characteristics to the material supplied by our collaborators in the UK (Prof. P.N. Hawkins, London). Using ^{125}I-labeled SAP as a tracer in mice with severe systemic AA-amyloidosis we have correlated the SPECT images with biodistribution analyses, micro-autoradiography and histology-based quantitation of amyloid and found a good concordance (Wall, J.S., *et al*. (2005) *Amyloid: The Journal of Protein Folding Disorders*, Vol.**12**, 146). *MicroCT/SPECT Software Developments:* This past year, we finished the implementation of a high-performance iterative algorithm for CT image reconstruction. We re-implemented the SPECT code to support simultaneous reconstruction of data from two or more detector heads. We used an in-house developed Monte Carlo code to determine that scatter adds a negligible amount of noise to our CT images. Preliminary SPECT studies were conducted that indicate that attenuation correction is warranted for I-125 based imaging, but not for Tc-99m. This work also seemed to indicate that scattering perhaps need not be corrected for. We also implemented an automated method for segmentation of the spleen in a contrast enhanced CT image, the idea being that this will facilitate quantifying the corresponding amyloid burden evident in a SPECT image.

Issues: This has been a transition year for the Partnership. After several key members of our research team (the engineering component) left ORNL the University Of Tennessee Graduate School Of Medicine invested in the construction of a new Preclinical Imaging Laboratory (PIL) at the University Of Tennessee Medical Center. Upon completion of the facility the microSPECT/CT scanner was moved to UT and is now housed in a room adjacent to a microPET P4 scanner. In addition, we have established animal and radiation protocols, waste stream mechanisms and SOP's for small animal experimentation in the new PIL. With the coincidental move by Prof. Steve Kennel (radiochemistry) from ORNL to UT we are now establishing a pipeline to evaluate new tracers for amyloid imaging as well as to validate the efficacy of new therapies for treating systemic amyloid disease. Furthermore, a growing collaboration with the UT College of Veterinary Medicine applying micro-imaging technologies to the clinical evaluation of client owned animals (pets) is very successful. Our BRP has catalyzed and facilitated the growth of a new center for micro-imaging and veterinary small animal evaluation that is undoubtedly going to contribute significantly to research and development at the University of Tennessee and associated Universities.

ISSUES

None.

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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 (NINDS)

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. In addition to 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, resulting from a transient temperature rise on the order of 10 mK upon absorption of 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.

STATUS OF RESEARCH AND PARTNERSHIP

Non-invasive molecular and functional imaging *in vivo* is promising for detecting and monitoring various physiological conditions in animals and ultimately humans. To this end, we

present a novel noninvasive technology, spectroscopic photoacoustic tomography (SPAT), which offers both strong optical absorption contrast and high ultrasonic spatial resolution. Optical contrast allows spectroscopic separation of signal contributions from multiple optical absorbers (e.g., oxy-hemoglobin, deoxy-hemoglobin, and a molecular contrast agent), thus enabling simultaneous molecular and functional imaging. SPAT successfully imaged with high resolution the distribution of a molecular contrast agent targeting integrin $\alpha_v\beta_3$ over-expressed in human U87 glioblastomas in nude mouse brains. Simultaneously, SPAT also imaged the hemoglobin oxygen saturation and the total hemoglobin concentration of the vasculature, which revealed hypoxia in tumor neo-vasculature. Therefore, SPAT can potentially lead to better understanding of the interrelationships between hemodynamics and specific biomarkers associated with tumor progression.

ISSUES

There was an accounting error in the first-year grant year. 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, we expect a delay in the delivery of the ultrasonic array.

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PROJECT TITLE: Structural MRI of Trabecular Bone for Therapy Response Monitoring

PARTNERS' NAMES AND AFFILIATIONS:

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

ABSTRACT

The chief manifestation of osteoporosis is the occurrence of fractures. Most osteoporotic fractures occur at skeletal locations rich in trabecular bone. Prevailing among these are the vertebrae, wrist and proximal femur. Hip fractures are the most debilitating among osteoporotic fractures in terms of morbidity and mortality. There is now strong evidence that the loss of bone mass is accompanied by a decline in the trabecular bone network's structural integrity. The impaired mechanical competence secondary to gonadal steroid depletion is caused by topological changes in the bone's architectural make-up, chief among which is fenestration of trabecular plates resulting in their conversion to rods and the latter's eventual disruption. Complementing antiresorptive treatment, new therapies have recently become available to treat the devastating consequences of severe bone loss with bone-forming (i.e. anabolic) drugs. It is not clear, however, whether such therapies are, in fact, able to reverse the disintegration of the trabecular network, and to what extent the structural changes differ from those induced by antiresorptive treatment. The chief objective of this project is to develop novel micro-MRI-based technology suitable for quantifying the structural and mechanical consequences of various forms of treatment of patients with metabolic bone disease. The aim is to apply this methodology to patients who are at high risk of fracture and who are treated either with 1-34 parathyroid hormone or alendronate. The overall hypothesis is that the new methodology will provide detailed insight into the structural manifestations of trabecular bone subjected to short-term drug treatment. The project consists of six specific aims involving the development, integration and evaluation of new methods involving data acquisition and reconstruction, motion correction, cryogenic RF coil technology, image processing and analysis, as well as image-based finite-element modeling of bone mechanical competence. We plan to address these goals in partnership with two external collaborators through inter-institutional subcontracts (Department of Electrical Engineering, Texas Center for Superconductivity, University of Houston, and Department of Biomedical Engineering, Columbia University).

STATUS OF RESEARCH AND PARTNERSHIP

Although we have not completed the first year of the project yet, the partnerships with external collaborators, the Department of Biomedical Engineering at Columbia University (Dr. Edward Guo) and the Department of Electrical Engineering, Texas Center for Superconductivity, University of Houston (Dr. Jarek Wosik), have been effective and progress has been made in both areas involving the partnerships. The chief objective of the research subcontracted to the University of Houston involves the development of 4- and/or 8-element superconducting (HTS) arrays, operating at 128 MHz (3 T), as a means to enable very high resolution

imaging of trabecular bone architecture that will provide detailed insight into the etiology of bone loss and its reversal through treatment with anabolic agents. The first eight months focused on development, fabrication and testing of 2- and 4-element arrays. Thus far we have achieved the following milestones: (1) We have designed and built circuitry for a high-Q 4-element array that achieves tuning, impedance matching and mutual inductance decoupling. (2) The electromagnetic modes of a 4-element array theoretically have been analyzed to learn how to *capacitively* compensate the coupling between array elements, since decoupling by overlap is unsuited for HTS elements. (3) Two- and 4-element copper arrays were constructed to operate at 64 and 128 MHz, made on PTFE printed circuit boards. Using finite-element modeling (HFSS, Ansoft), coils were designed to be sensitive to RF magnetic fields but insensitive to RF electric fields by patterning double-sided structures in either copper or HTS (YBCO) films. (4) A single-element double-sided HTS coil (YBCO on sapphire), integrated with a small G-10 liquid nitrogen cryostat, was constructed for use at 64 MHz.

Significant progress has also been made at the Columbia site. Here, the objective is to develop, test and apply novel computational biomechanics approaches as a means to predict the temporal changes in bone strength in response to treatment on the basis of serially acquired micro-MR images as input. Since in vivo trabecular bone images have limited SNR, such a goal poses significant challenges. During year 1 of the project the following milestones have been accomplished: (1) A computational approach based on finite element modeling has been developed and implemented by Dr. Guo and co-workers for predicting trabecular bone mechanical behavior. The computational algorithm has been validated rigorously with various anisotropic material models indicating that the algorithm can reproduce the exact full anisotropic elastic matrix. (2) High-resolution micro-CT images were acquired from human trabecular bone specimens of various anatomic sites. These images were subsequently resampled to lower resolution along with addition of noise for the purpose of mimicking the in vivo situation to determine the dependence of the derived Young's moduli on resolution and SNR. (3) In vivo micro-MRI data of the distal tibia acquired previously as part of a different project to examine the effect of treatment of 10 hypogonadal men with testosterone were subjected to full FE analysis after appropriate processing to a voxel size of 68x68x102 μm^3. The results showed that commensurate with improved structural integrity, the outcome of 24 months of treatment was an increase in the elastic modulus for longitudinal uniaxial loading ($p=0.04$) and shear moduli ($p=0.02$).

Activities at the host lab focused mainly on data acquisition and data management to improve image quality and reproducibility and allow effective tracking and archiving of patient and image data. These endeavors included: (1) The further development and testing of new approaches to compensation for image degradation as a result of patient movement during the scan. Three different avenues were pursued within the framework of both radial and cartesian sampling strategies: (a) A substantially improved 3D FLASE pulse sequence was designed and implemented at 1.5T. The new sequence uses out-of-slab navigators for more accurate sensing of translational displacements and incorporates measures to essentially eliminate the possibility of aliasing, ghosting, or banding artifacts which have in the past been difficult to suppress. (b) Experiments were performed with a 3D hybrid radial pulse sequence that allows for retrospective motion correction without the need for acquiring additional navigator echoes. (c) Navigator correction for translational displacements was combined with the autofocus technique correcting for rotational motion, showing significantly improved image sharpness. (2) We have streamlined and further automated the entire processing cascade, ranging from volume-of-interest selection to parameter extraction in view of the long-term goal of achieving large-scale processing of clinical data for therapy response monitoring. Our new automatic algorithms eliminate the need for any user intervention during image processing and parameter extraction (including the 3D registration of baseline and follow-up scans in longitudinal studies). (3) We have partially implemented a password-protected web-based database and management system for handling patient and multi-modality (MRI, DEXA, pQCT) data accessible to the qualified user. The system provides access to a custom-built 3.2 TB RAID server used for long-term storage of source data and processed images. As we pursue the acquisition of higher resolution 3D images, the image file sizes will increase significantly, and computing efficiency will become more of an issue. The password-protected web server will allow custom processing to be performed on each individual user's local machine, opening the possibility of highly-efficient parallel processing of data.

ISSUES

None.

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PROJECT TITLE: Methods in Molecular Imaging and Targeted Therapeutics

PARTNER'S NAMES AND AFFILIATIONS:
Kereos, Inc. (St Louis, MO.); U of Missouri (Columbia, MO.)

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

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

Translational activities: Exceptional progress has been made by Kereos in finalizing the formulation of a proprietary gadolinium chelate for clinical trials. During the past 12 months, Kereos has completed optimizing the $\alpha_v\beta_3$ targeting ligand, adopting alternative phospholipid and linker chemistries to solve stability problems that arose upon thermal sterilizations, and to improve process efficiencies. Synthesis of this optimized targeting ligand has been successfully demonstrated at commercial-scale and more than 1 gram of material has been transferred to Washington University. In parallel, Kereos has also developed and validated analytical methods, as well as cell adhesion assays, for this ligand, both as an isolated material and coupled to perfluorocarbon emulsions. Bioanalytical methods for detection of material in human and animal biological samples have been developed that will be critical for clinical trials. Finally GMP production capabilities have been developed that will provide material for clinical trials int eh near future for detection of angiogenesis.

Kereos has proposed and synthesized a novel Gd-DOTA-PE which is now being provided to Washington University. Transmetallation studies have shown this to be a highly stable chelator (note: less than 1% transmetalation!), and measurements in collaboration with the Wickline/Lanza laboratories indicate ionic relaxivities in excess of that for the previous agents proposed for use in this research (Gd-MeO-DOTA-PE and Gd-DTPA-BO): see Figure 1. More than 2 grams of material have been transferred to Washington University. In parallel, Kereos has also developed and validated analytical/bioanalytical methods for this Gd-chelate.

Finally, Kereos has acquired the license for an alternative small molecule ligand from our partner Bristol-Myers Squibb Medical Imaging, which will be complementary to $\alpha_v\beta_3$ for characterizing angiogenesis: $\alpha_5\beta_1$ integrin. Both this ligand and the $\alpha_v\beta_3$ small molecule ligand will employ the same strategy fo complexing

with nanoparticles and should be ready by Q3 2006 for MRI trials in ApoE -/- cholesterol fed mice for imaging atherosclerosis at 11.7T

Academic activities: We have spent a good deal of effort transferring imaging experiements to the new 11.7T horizontal bore imager that was installed last year in the Mallinckrodt East Building. This technology will propel the fluorine imaging projects and the high resolution work required for both diagnosis and drug delivery. Substantial effort has been put into coil development, pusle programming, and monitoring to faciliate the transition from the current 4.7T systems. As a consequence, we have had a large number of abstracts accepted for oral (7) and poster (2) presentation at the ISMRM meeting in Seattle in May 2006 that represent our progress (See references ??). A brief description of three major advances follows.

a. *ApoE-/- mouse fluorine imaging/spectroscopy at 11.7T:* We report the development and implementation of a novel method for definitively detecting plaque angiogenesis associated with atherosclerosis , in a well-described murine model of atherosclerosis. Because fluorine is not abundant in living tissue, perfluorocarbon-based targeted nanoparticles represent an excellent candidate agent for characterizing pathological molecules causative of atherosclerosis with the use of MRI and MRS. We have demonstrated that it is possible to detect angiogenesis with perfluorocarbon particle formulations, and further, that the method is sensitive enough to detect spatial variations in disease extent based on fluorine signal strength from targeted particles (Fig. 2). Because the fluorine spectrum is theoretically quantitative with respect to the number of nanoparticles bound (and thus molecular epitopes present), this method may permit localized fluorine spectroscopy to delineate the extent of plaque angiogenesis in different regions of the mouse aorta in vivo.

b. *Fluorine angiography at 1.5T:* While the current gold standard for coronary imaging is X-ray angiography, evidence is accumulating that it may not be the most sensitive technique for detecting unstable plaque. Other imaging modalities, such as MRI, can be used for plaque characterization, but suffer from long scan and reconstruction times for determining regions of stenosis. We have developed an intravascular fluorinated contrast agent that can be used for angiography with MRI at clinical field strengths (1.5 T). This liquid perfluorocarbon nanoparticle contains a high concentration of fluorine atoms that can be used to generate contrast on 19F MR images without any competing background signal from surrounding tissues. By using a perfluorocarbon with 20 equivalent fluorine molecules, custom-built RF coils, a modified clinical scanner, and an efficient steady-state free procession sequence, we demonstrate the use of this agent for angiography of small vessels in vitro, ex vivo, and in vivo. The surprisingly high signal generated with very short scan times and low doses of perfluorocarbon indicates that this technique may be useful in clinical settings when coupled with advanced imaging strategies. A patent has been submitted for this procedure.

c. *Endothelial precursor cell labeling and tracking at 1.5T and 11.7T:* Endothelial precursor cell tracking is critical for understanding angiogenesis in animal models of plaque growth. We have developed a new method for labeling and tracking such cells that can be applied to human stem cells obtained from cord blood. Although conventional proton (^{1}H) magnetic resonance imaging (MRI) has been employed for noninvasive *in vivo* stem/progenitor cell tracking, we now demonstrate that fluorine (^{19}F) MRI of cells labeled with multiple types of liquid perfluorocarbon (PFC) nanoparticles produces unique and sensitive cell markers. Mononuclear cells harvested from human umbilical cord blood were grown under proendothelial conditions and labeled with fluorescent nanoparticles composed of two distinct PFC cores. Stem/progenitor cells ($CD34^{+}CD133^{+}CD31^{+}$) readily internalized PFC nanoparticles without aid of adjunctive labeling techniques. The cells were rapidly imaged (~7min) using the ^{19}F nanoparticle signal at both 11.7T and 1.5T *in vitro*, and in mouse skeletal muscle *in situ (see Figure 3)*. PFC nanoparticles provide a unique cellular signal, enable spatial cell localization with ^{19}F MR imaging, and permit quantification and detection of multiple fluorine signatures via ^{19}F MR spectroscopy. This method should facilitate longitudinal investigation of cellular events *in vivo* such as stem/progenitor cell localization, implantation, and differentiation. A patent has been submitted for this procedure

ISSUES

none

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

PARTNERS' NAMES AND AFFILIATIONS:

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institute of Neurological Disorders and Stroke (NINDS)

ABSTRACT

Signals from the brain can provide a new communication channel, a brain-computer interface (BCI), for people with severe neuromuscular disorders such as amyotrophic lateral sclerosis (ALS), brainstem stroke, cerebral palsy, and spinal cord injury. BCI technology can allow people who are completely paralyzed, or "locked in," to express wishes to caregivers, use word processing programs, access the Internet, or even operate neuroprostheses.

Up until 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 people 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 BRP project are: (1) to develop a flexible general-purpose BCI system that can incorporate any of the relevant signals, analyses, and operating formats and that 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 that has clear practical value for those with motor disabilities.

STATUS OF RESEARCH AND PARTNERSHIP

The past year has seen substantial progress. The program continues to focus on: development and dissemination of BCI2000, our general-purpose BCI system; optimizing use of this system to enable people with and without disabilities to use EEG-based BCI methods (sensorimotor (i.e., mu and beta) rhythms and the

P300 evoked potential) and related electrocorticographic(ECoG)-based BCI methods; and clinical implementation and validation of this BCI technology. Major progress includes:

(1) Further improvements and extensions of BCI2000. These include: interfacing the system with off-the-shelf communication aids (such as predictive spelling programs); and developing related software tools (e.g., the offline analysis program MARIO and a new BCI2000 plug-in for real-time visualization of brain function called SIGFRIED (SIGnal modeling For Real-time Identification and Event Detection). To date, BCI2000 has been adopted by more than 80 laboratories around the world and is rapidly becoming the standard in the field. It is supported by a very energetic user group and an active website.

(2) Further improvements in EEG-based multidimensional and sequential movement control. With these refinements, trained users can now master two-dimensional movement to a goal (i.e., reach) followed by selection of the goal (i.e., grasp). Two independent EEG control signals move a cursor to one of multiple possible targets in two dimensions, and a third selects or rejects the target. In studies to date, users, including those with spinal cord injury, achieve >90% accuracy.

(3) The Atlanta partner continues to provide BCI2000 software adaptations including improvements to the P300 program and the 3-D application. This partner also provided the initial programming and testing for BCI2000-based SSVEP control.

(4) Together with the Tuebingen partner, we are implementing and evaluating clinical use of the laboratory-proven BCI systems described above. To do this, we have significantly reduced the complexity of the necessary hardware and BCI2000 configuration to produce a BCI2000-based home system. We have begun testing this system for everyday use by people severely disabled by ALS. The first such user is a NIH-funded scientist with ALS who can only move his eyes. He has had the BCI home system for 5 months and reports that he much prefers it to his eye-gaze communication system. He now uses the BCI system 6-8 hrs/day for e-mail and other purposes.

(5) With our partners at Washington University in St. Louis and the University of Washington in Seattle, we continue to explore the BCI use of electrocorticographic (ECoG) activity recorded from the cortical surface. We recently completed a study that demonstrated that ECoG can be used to decode position and velocity of two-dimensional hand movements, and showed that the accuracy of this decoding is comparable to that previously described for signals recorded using intracortical microelectrodes. We also completed a study that demonstrated that ECoG recorded can support two-dimensional movement control.

(6) Finally, to continue to support and develop the field of BCI research, we have hosted the 3rd International BCI Meeting, edited a special BCI issue of the IEEE Transactions of Neural Systems and Rehabilitation comprised of 31 peer-reviewed articles from the meeting, and hosted the first BCI2000 Workshop.

ISSUES

The work of the BRP is progressing very well. The most critical issues concern the appropriate allotment of the Partnership's time and effort among the important opportunities and responsibilities now presenting themselves. Chief among these are: the continued dissemination and support of the general-purpose BCI system BCI2000; the validation of BCI clinical applications and their dissemination to the user populations most in need of them; and continued exploration and progressive clinical implementation of EEG/ECoG-based BCI technology.

The continued maintenance and support of the BCI2000 software now in use by more than 80 research laboratories around the world is the purpose of a newly funded RO1 application. The development of an appropriate mechanism for ensuring that BCI home systems can be provided (along with technical support) to those who most need them is the subject of current discussions. It is likely that a non-profit entity will eventually be established to serve this function. The primary work of the BRP will continue to focus on improvement of EEG-based BCI communication and control, and on concurrent development of related ECoG-based BCI methods, which, like EEG-based methods, will eventually come to clinical implementation and validation.

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PROJECT TITLE: Engineering Development of a Chronic Retinal Implant

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GRANTING NIH INSTITUTE/CENTER: National Eye Institute (NEI)

ABSTRACT
The broader purpose of this project is to develop a radically altered design for a wireless retinal prosthesis to restore some useful level of vision to patients blind with retinitis pigmentosa or macular degeneration. The specific goals for this proposal are to: redesign the implant chip for enhanced functionality, to permanently coat the stimulating microelectrode array in biocompatible materials, to permanently shield the microelectronics from saline by placing them in a custom designed hermetic package designed for implantation within the eye socket, and to surgically test prototypes in the dog eye.

STATUS OF RESEARCH AND PARTNERSHIP
Our BRP was initiated June 1, 2005. We are happy to report significant progress in the development of biocompatible coatings for microelectrode arrays, the development of a hermetic package with wire-feedthroughs for long-term implantation, and the refinement of our surgical techniques in animal models. We have also designed, built and tested improved data and power transmitters in preparation for the first chronic animal tests. We have also developed a reliable, expanded picoampere soak-test system to test for minute amounts of saline leakage during prolonged saline immersion. Our biggest accomplishment is that we have developed, somewhat ahead of schedule, a working, wireless first-generation implant.

ISSUES

We have organized this project around an engineering manager who takes responsibility for task assignment, priorities and deadlines, and resource management. The team members are comfortable and cooperative with one another, and the design and testing of prototypes are developing as planned. But the progress is slower than we want. The primary issue is that neither MIT nor Cornell has the facilities to do all the special wire-bonding and other assembly steps that are required for the implant. Therefore these capabilities are contracted out to vendors such as Engent, Inc. and Valtronic, Inc. that specialize in these areas. But we are relatively low-budget, small-volume customers, and therefore often have difficulty getting rapid attention and good yield from these companies.

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PROJECT TITLE: Robot-Assisted Platform for Intratumoral Delivery

PARTNERS' NAMES AND AFFILIATIONS:
Nanyang Technological University (Singapore), RTek Medical Systems (Pittsford, NY)

GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI)

ABSTRACT

Intratumoral therapies of prostate cancer include the delivery of brachytherapy or ablation energy sources, or drug delivery, 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. 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 replanning and re-optimization of therapy delivery. The integrated RAPID system 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. 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, multimodality capabilities of the RAPID system will be continually exploited towards an integrated diagnosis and therapy engine for localized cancers of solid organs.

STATUS OF RESEARCH AND PARTNERSHIP

In the current grant year, the BRP accomplished the major implementation tasks of the project. These include detailed design of the robotic hardware system, fabrication, software and control system development, and integration testing. In addition, ongoing experimental research on increasing targeting accuracy, minimizing tissue deformation and organ motion has yielded important control strategies for implementation in the next step.

The robotic system consists of a 9 DOF (degree-of-freedom) Positioning Module and a 7 DOF Surgery Module. The positioning module consists of a 3 DOF Cart and a 6 DOF Platform. The Surgery Module is mounted on the Platform. At the starting of the procedure, by maneuvering the cart the whole system is brought to a suitable location for convenient access to the patient. Then by adjusting the Passive Platform, the Surgery Module is positioned and orientated at the desired location. The cart can move omni-directionally on the floor, and includes a floor locking mechanism.

The Surgery Module consists of a 2 DOF ultrasound probe Driver, a 3 DOF Gantry, a 2 DOF Needle Driver and a Seed Pusher. The US probe can be translated and rotated separately by two DC servo motors fitted with encoders and gear boxes. This enables imaging in transverse plane as well as in sagittal plane, thus providing capability for improving 3D planning, image guidance and verification. The needle driving module is connected to the positioning platform by the gantry. The gantry has two translational motions (x- & y- direction) and one rotational motion (pitch). The motions are achieved by DC servo motors and optical encoders fitted with the motors. The 3DOF motions of the gantry can position and orient the needle at a desired location on the patient's perineum with greater freedom due to the absence of a physical template used in conventional brachytherapy; thus can reach any clinically significant target locations in the prostate. Once the needle is positioned at the desired location close to the perineum, the needle driver inserts the needle into the patient. The motions of the US probe driver and the rest of the Surgery Module (gantry and needle driver) are decoupled by making two separate open kinematic chains attached to the same positioning platform. Though both the kinematic chains are attached to the same mounting plate for better rigidity and ease of calibration, they are dynamically decoupled.

The needle consists of a hollow cannula and a solid stylet that are driven separately by two DC servo motors. Since the stylet and cannula motions are concentric they essentially provide single DOF. However, the cannula can be rotated continuously (spinning) or partially using another tiny DC motor. Thus, with 3 motorized motions the needle driver actually has 2DOF. Both the stylet and the cannula are driven from the back (they are pushed); therefore there is no chance of slipping.

To measure and monitor force profiles during the operational procedures, we have installed two single-axis force sensors each at the proximal ends of the stylet and cannula, and one six-axis force-torque sensor at the distal end of the cannula. Monitoring of these forces is useful in detecting pubic arch interference (PAI) and will help in assessing needle bending.

To evaluate the system's repeatability and accuracy, we are in the process of carrying out the System Integration Test Plan. The system was run in a fixed pattern for about 2 hours at a time. An 18 gauge beveled-tip brachytherapy needle was inserted into a graph paper pasted on a block of foam. There were a total of 16 penetration locations in a 60mm x 60mm area. The gantry moved the needle driver in a sequence so that the mechanical systems go through back and forth motion to experience backlash/slack errors in the motion transfer trains/linkages. After a single penetration at each location the needle was moved to the next location, and this was repeated for 100 times. Additionally, needle placement accuracy was tested in phantoms of varying hardness and consistency. Preliminary assessment of repeatability and accuracy is highly encouraging.

Experimental results reveal that velocity modulation, especially rotation, significantly improves needle insertion and targeting accuracy. Reduction in force also minimizes tissue/organ deformation and target deflection, and thus is expected to improve therapeutic delivery accuracy.

As of this report, the RAPID software system is over 90% completed, with most of the planned functional modules fully implemented. The software is currently capable of 1) handling the patient record; 2) scanning the prostate volume in both transverse and sagittal modes; 3) building the 3D anatomical model of prostate anatomy; 4) calculating and re-calculating the dosimetry for the given prostate model using inverse planning and hybrid dosimetry; 5) controlling the robot motion in all phases of the procedure; 6) executing *loaded* plan; 7) handling some of the unplanned stop scenarios.

ISSUES

There has been no issue in the grant year.