FOURTH ANNUAL BIOENGINEERING RESEARCH PARTNERSHIP GRANTEE MEETING

National Institutes of Health
Bioengineering Consortium

Bethesda Hyatt
Bethesda, Maryland

July 29-30, 2004

Bioengineering Consortium

National Institutes of Health
Bethesda, MD 20892

Welcome to the Fourth Annual Bioengineering Research Partnership Grantee Meeting.

This February marked the seventh anniversary of the Bioengineering Consortium (BECON) which provides a focus for biomedical engineering research and training activities at the National Institutes of Health (NIH). Active participation by all NIH centers, institutes, and offices and other Federal agencies has facilitated the realization of substantial benefits from the application of engineering, physical, and computational science principles and techniques to address problems in biology and medicine. The importance of this field is reflected by the steady increase in total annual funding for bioengineering research over the past seven years and the establishment of the National Institute for Biomedical Imaging and Bioengineering at the NIH.

To facilitate the development of the field of bioengineering, the BECON has coordinated trans-NIH initiatives aimed at encouraging and supporting multi-disciplinary and integrative approaches to biomedical research and training. One of the most successful and visible of these research 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 between the biomedical sciences and the allied disciplines that can provide significant advances for improving human health. To date, over 120 BRP awards have been made for a total investment of about $500 million by sixteen NIH research institutes and centers.

This meeting is the fourth time that the BRP grantees, BECON members, and NIH institute/center representatives will gather to discuss research projects, bioengineering issues, and the program in general. This is the first meeting that includes poster presentations and the “open mic” feature. As always, your perspectives and suggestions concerning partnership experience and management, program efficacy, bioengineering research and training needs, and future BRP grantee meetings are welcome and solicited. Also, please take this opportunity to meet with your NIH institute/center representative to discuss progress and concerns for your project.

I hope that the BRP Grantee Meeting is valuable and enjoyable to you. All the BECON members and NIH program staff look forward to your participation and comments.

Dr. Daniel Sullivan, Chair
Bioengineering Consortium

Welcome to the Fourth Annual
BIOENGINEERING RESEARCH PARTNERSHIP
Grantee Meeting

AGENDA

Thursday, July 29, 2004

8:30 AM	**Registration and Poster Set-up**
9:30 AM	**Welcome and Orientation** (Waterford Room) *Dan Sullivan (NCI) and Richard Swaja (NIBIB)*
9:45 AM	**Grantee Presentations** (Waterford and Cartier/Tiffany Rooms)
11:15 AM	**Lunch Presentation** (Waterford Room) Bioengineering and the Whitaker Foundation *Peter Katona (President, Whitaker Foundation)*
12:45 PM	**NIH Update** (Waterford Room) Current Issues at the NIH: *Norka Ruiz Bravo (OER)* Technology Transfer at the NIH: *John Kim (OTT)* NIH Roadmap: *Belinda Seto (NIBIB)* Review of Bioengineering Proposals: *Jean Sipe (CSR)*
2:00 PM	**Break**
2:15 PM	**Poster Session** (Haverford Room) and **NIH Staff Discussions** (Waterford Room)
4:15 PM	**Open Mic and Discussion** (Waterford Room)
5:00 PM	**Adjourn**

Friday, July 30, 2004

7:15 AM	**Light Refreshments**
8:00 AM	**Welcome and Orientation** (Waterford Room) *Dan Sullivan (NCI) and Richard Swaja (NIBIB)*
8:15 AM	**Grantee Presentations** (Waterford and Cartier/Tiffany Rooms)
9:45 AM	**Break**
10:15 AM	**Grantee Presentations** (Waterford Room)
10:45 AM	**Open Mic and Discussion** (Waterford Room)
11:30 AM	**Summary**
11:45 AM	**Adjourn**

Attendees

Jeffry Alger, Ph.D.
Department of Radiological Sciences
University of California, Los Angeles
T: 310-206-3344
jralger@ucla.edu

Kamran Badizadegan, M.D.
Department of Pathology
Massachusetts General Hospital
T: 617-726-6710
kbadizadegan@partners.org

Alfred Bahnson, Ph.D.
Automated Cell, Inc.
T: 412-826-5205 ext. 5219
abahnson@automatedcell.com

Lucinda Baker, Ph.D.
Department of Biokinesiology & Physical Therapy
University of Southern California
T: 323-442-2900
llbaker@usc.edu

Byron Ballou, Ph.D.
Molecular Biosensor and Imaging Center
Carnegie Mellon University
412-268-4779 ext. 4986
bbal@andrew.cmu.edu

Michael Berns, Ph.D.
Department of Biomedical Engineering
Beckman Laser Institute
T: 949-824-6291
mberns@bli.uci.edu

Nicolas Bolo, Ph.D.
Brain Imaging Center
McLean Hospital – Harvard Medical School
T: 617-855-2596
nbolo@mclean.harvard.edu

John Boone, Ph.D.
Research Imaging Center
University of California Davis Medical Center
T: 916-734-3158
john.boone@ucdmc.ucdavis.edu

Michael Bottlang, Ph.D.
Legacy Clinical Research & Technology Center
T: 503-413-5457
mbottlang@biomechresearch.org

Emery Brown, M.D., Ph.D.
Department of Anesthesia and Critical Care
Massachusetts General Hospital
T: 617-726-7487
brown@neurostat.mgh.harvard.edu

Thomas Buchanan, Ph.D.
Department of Mechanical Engineering
University of Delaware
T: 302-831-2410
buchanan@me.udel.edu

Zhongping Chen, Ph.D.
Beckman Laser Institute
University of California, Irvine
T: 949-824-1247
zchen@laser.bli.uci.edu

Alfred Cheung, M.D.
Dialysis Program
University of Utah
T: 801-581-6427
alfred.cheung@hsc.utah.edu

Shu Chien, M.D., Ph.D.
Department of Bioengineering
University of California, San Diego
T: 858-534-5195
shuchien@ucsd.edu

E. Chiocca, M.D., Ph.D.
Department of Neurological Surgery
Ohio State University Medical Center
T: 614-293-9312
chiocca-1@medctr.osu.edu

Robert Clarke, Ph.D., D.Sc.
Departments of Oncology and
Physiology & Biophysics
Georgetown University School of
Medicine
T: 202-687-3755
clarker@georgetown.edu

Mark Clemens, Ph.D.
Department of Biology
University of North Carolina at
Charlotte
T: 704-687-4040
mgclemen@email.uncc.edu

Edward Crandall, M.D., Ph.D.
Department of Medicine
University of Southern California
T: 323-226-7923
ecrandal@usc.edu

Anders Dale, Ph.D.
Department of Radiology
Harvard University
T: 617-724-9771
dale@nmr.mgh.harvard.edu

Peter Davies, Ph.D.
Institute for Medicine & Engineering
University of Pennsylvania
T: 215-573-6813
pfd@pobox.upenn.edu

Pedro del Nido, M.D.
Department of Cardiovascular Surgery
Childrens Hospital Boston
pedro.delnido@tch.harvard.edu

Carlo De Luca, Ph.D.
Neuromuscular Research Center
Boston University
T: 617-353-9756
cjd@bu.edu

Peter Doerschuk, M.D., Ph.D.
School of Electrical and Computer
Engineering
Purdue University
T: 765-494-1742
doerschu@ecn.purdue.edu

Jonathan Dordick, Ph.D.
Department of Chemical and Biological
Engineering
Rensselaer Polytechnic Institute
T: 518-276-2899
dordick@rpi.edu

Mark Doyle, Ph.D.
Allegheny General Hospital
T: 412-359-4243
mdoyle@wpahs.org

James Duncan, Ph.D.
Department of Diagnostic Radiology
Yale University
T: 203-785-2427
james.duncan@yale.edu

Gareth R. Eaton, Ph.D.
Department of Chemistry and
Biochemistry
University of Denver
T: 303-871-2980
gareth.eaton@nsm.du.edu

Michael Feld, Ph.D.
George R. Harrison Spectroscopy
Laboratory
Massachusetts Institute of Technology
T: 617-253-7700
msfeld@MIT.edu

John Frangos, Ph.D.
La Jolla Bioengineering Institute
T: 858-456-7505
frangos@ljbi.org

Jeffrey Fredberg, Ph.D.
Department of Environmental Health
Harvard School of Public Health
T: 617-432-0198
jfredber@hsph.harvard.edu

Brent French, Ph.D.
Department of Biomedical Engineering
University of Virginia
T: 434-924-5728
bf4g@virginia.edu

Lisa Gallagher, M.S.
Department of Mechanical Engineering
SUNY Binghamton
T: 607-777-4821
lisag@binghamton.edu

Charles Gilbert, M.D., Ph.D.
Department of Neurobiology
Rockefeller University
T: 212-327-7670
Gilbert@mail.rockefeller.edu

Robert Greenberg, M.D., Ph.D.
Second Sight Medical Products
T: 818-833-5000
bob@2-sight.com

Jens Gregor, Ph.D.
Department of Computer Science
The University of Tennessee
jgregor@cs.utk.edu

Henry Halperin, M.D., M.A.
Johns Hopkins University
T: 410-955-2412
hhalper@jhmi.edu

David Haake, M.D.
Infestious Diseases Section
VA Greater L.A. Healthcare System
dhaake@ucla.edu

Bruce Hasegawa, Ph.D.
Department of Radiology
UCSF Physics Research Laboratory
T: 415-353-9472
bruceh@itsa.ucsf.edu

Kip Hauch, Ph.D.
Department of Engineered Biomaterials
University of Washington
T:206-543-0289
hauch@u.washington.edu

Ronald Hirschl, M.D.
Department of Surgery
Children's Hospital
University of Michigan
rhirschl@umich.edu

Eric Hoffman, Ph.D.
Department of Radiology
University of Iowa College of Medicine
T: 319-356-1381
eric-hoffman@uiowa.edu

Raymond Houck, M.S.
Automated Cell, Inc.
T: 412-826-5250
rhouck@automatedcell.com

David Huang, M.D., Ph.D.
Doheny Eye Institute
University of Southern California
T: 323-442-6710
davivhuang@mindspring.com

Jay Humphrey, Ph.D.
Department of Biomedical Engineering
Texas A&M University
T: 979-845-5558
jhumphrey@tamu.edu

Marcos Intaglietta, Ph.D.
Department of Bioengineering
University of California, San Diego
T: 858-534-4275
mintagli@ucsd.edu

Rakesh Jain, Ph.D.
Massachusetts General Hospital
Harvard Medical School
T: 617-726-4083
jain@steele.mgh.harvard.edu

Steven Jaques, Ph.D.
Departments of Biomedical Engineering and Dermatology
Oregon Health and Science University
T: 503-748-1512
sjaques@bme.ogi.edu

John Johnson, Ph.D.
The Scripps Research Institute
Department of Molecular Biology
T: 858-784-9705
jackj@scripps.edu

Ken Kasza, Ph.D.
Argonne National Laboratory
kasza@anl.gov

Saeed Khan, Ph.D.
Department of Pathology, Immunology and Laboratory Medicine
University of Florida
T: 352-392-3574
khan@pathology.ufl.edu

Nikolay Kharin, Ph.D.
Department of Biomedical Engineering
The Cleveland Clinic Foundation
T: 216-445-3577
kharinn@bme.ri.ccf.org

Wolff Kirsch, M.D.
Loma Linda University Neurosurgery Center for Research, Training, & Education (Laboratories) and Biochemistry Department
wkirsch@som.llu.edu

Fred Koller, Ph.D.
Oncosis
T: 858-450-7063
fkoller@oncosis.com

Michelle LaPlaca, Ph.D.
Georgia Institute of Technology
michelle.laplaca@bme.gatech.edu

Simon Levine, Ph.D.
Departments of Physical Medicine and Rehabilitation and Biomedical Engineering
University of Michigan
T: 734-936-7170
silevine@med.umich.edu

Julie Yi-Shuan Li, Ph.D.
Department of Bioengineering
University of California, San Diego
T: 858-822-1313
jli@bioeng.ucsd.edu

Joseph Liao, M.D.
Department of Urology
David Geffen School of Medicine at UCLA
jcliao@mednet.ucla.edu

Charles Lin, Ph.D.
Massachusetts General Hospital
Harvard Medical School
T: 617-724-3957
lin@helix.mgh.harvard.edu

CC Ling, Ph.D.
Department of Medical Physics
Memorial Sloan Kettering Cancer Center
T: 212-639-8301
lingcc@mskcc.org

Brian Litt, M.D.
Department of Neurology
University of Pennsylvania
littb@mail.med.upenn.edu

Frederick Lizzi, ENG.Sc.D.
Riverside Research Institute
Biomedical Engineering Laboratories
T: 212-502-1774
lizzi@rrinyc.org

Gerald Loeb, M.D.
Institute for Biomedical Engineering
University of Southern California
T: 213-821-1112
gloeb@usc.edu

Richard Long, Ph.D.
Department of Blindness and Low Vision Studies
Western Michigan University
T: 269-387-3451
richard.long@wmich.edu

Duncan Maitland, Ph.D.
Medical Physics and Biophysics Division
Lawrence Livermore National Laboratory
T: 925-423-6697
Maitland1@llnl.gov

Roger Mark, M.D., Ph.D.
Division of Health Sciences and Technology
Massachusetts Institute of Technology
T: 617-253-7818
rgmark@mit.edu

Michael Matthews, Ph.D., P.E.
Department of Chemical Engineering
University of South Carolina
T: 803-777-0556
matthews@engr.sc.edu

Andrew Maudsley, Ph.D.
University of Miami School of Medicine
T: 305-243-8080
amaudsley@med.miami.edu

Larry McIntire, Ph.D.
The Wallace H. Coulter Department of Biomedical Engineering at Georgia Tech and Emory University
larry.McIntire@bme.gatech.edu

Timothy McKnight, M.S.
Condensed Matter Sciences Division
Oak Ridge National Laboratory
T: 865-574-5681
mcknightte@ornl.gov

David Meaney
Department of Bioengineering
University of Pennsylvania
T: 215-573-3155
dmeaney@seas.upenn.edu

Srboljub Mijailovich, Ph.D.
Department of Environmental Health
Harvard School of Public Health
T: 617-432-4814
smijailo@hsph.harvard.edu

Ronald N. Miles, Ph.D.
Department of Mechanical Engineering
State University of New York at Binghamton
T: (607) 777-4038
miles@binghamton.edu

Wayne Mitzner, Ph.D.
Johns Hopkins Bloomberg School of Public Health
T: 410-614-5446
wmitzner@jhsph.edu

PH Peckham, Ph.D.
Department of Biomedical Engineering
Case Western Reserve University
T: 216-778-3480
pxp2@case.edu

Douglas Pedersen, Ph.D.
Department of Orthapaedic Biomechanics
University of Iowa

Eli Peli, M.Sc., O.D.
Schepens Eye Research Institute
Harvard Medical School
T: 617-912-2597
eli@vision.eri.harvard.edu

Arkady Pertsov, Ph.D.
Department of Pharmacology
SUNY Upstate Medical University
T: 315-464-7986
pertsova@upstate.edu

Richard Rabbitt, Ph.D.
Department of Bioengineering
University of Utah
T: 801-581-6968
r.rabbitt@m.cc.utah.edu

Perry Renshaw, M.D., Ph.D.
McLean Brain Imaging Center
Harvard Medical School
T: 617-855-3750
perry@mclean.harvard.edu

James Russell, Ph.D.
Memorial Sloan Kettering Cancer Center
russellj@MSKCC.ORG

Grady Rylander, M.D.
Department of Biomedical Engineering
University of Texas at Austin
T: 512-471-1995
rylander@mail.utexas.edu

Chris Sackellares, M.D.
Department of Neurology
University of Florida
T: 352-376-1611 ext. 6584
sackellares@mbi.ufl.edu

David J. Sahn, M.D.
Pediatrics (Cardiology)
Oregon Health & Science University
T: (503) 494-2191
sahnd@ohsu.edu

Guy Salama, Ph.D.
Department of Cell Biology and Physiology
University of Pittsburgh, School of Medicine
T: 412-648-9354
gsalama+@pitt.edu

William Shain, Ph.D.
Wadsworth Center
T: 518-473-3630
shain@wadsworth.org

Svetlana Shavalovskaya, Ph.D., Sc.D.
Institute for Physical Research and Technology
Iowa State University
T: 515-294-1293
shabalov@ameslab.gov

Larry Sklar, Ph.D.
Cancer Research Facility
University of New Mexico Health Sciences Center
lsklar@salud.unm.edu

Michael Smith, Ph.D.
Department of Radiology
Pennsylvania State University
T: 717-531-6069
mbsmith@psu.edu

William Smith, D.ENG, P.E.
Department of Biomedical Engineering
The Cleveland Clinic Foundation
T: 216-445-9334
wasmith@bme.ri.ccf.org

Alan Snyder, Ph.D.
Institute for Biomedical Engineering
Pennsylvania State University
T: 717-531-7068
asnyder@psu.edu

Steven Soper, Ph.D.
Department of Chemistry
Louisiana State University
T: 225-578-1527
chsope@lsu.edu

Sankararaman Suryanarayanan, M.S.
Research Associate
Emory University
Winship Cancer Institute
ssuryan@emory.edu

William Robert Taylor, M.D., Ph.D.
School of Medicine
Emory University
T: 404-727-8921
wtaylor@emory.edu

Wendy Thomas, Ph.D.
Department of Bioengineering
University of Washington
wendyt@u.washington.edu

Antoni Tomsia, Ph.D.
Materials Science Division
University of California at Berkeley
T: 510-486-4918
aptomsia@lbl.gov

Robert Tranquillo, Ph.D.
Department of Biomedical Engineering
University of Minnesota
T: 612-625-6868
tranquil@mozart.cems.umn.edu

Phil Troyk, Ph.D.
Pritzker Institute of Biomedical Science and Engineering
Illinois Institute of Technology
T: 312-567-5324
troyk@iit.edu

Akira Tsuda, Ph.D.
Physiology Program
Harvard School of Public Health
T: 617-432-0127
atsuda@hsph.harvard.edu

Tuan Vo-Dinh, Ph.D.
Oak Ridge National Laboratory
T: 865-574-6249
vodinht@ornl.gov

Alan Waggoner, Ph.D.
Molecular Biosensor and Imaging Center
Carnegie Mellon University
T: 412-268-3456
waggoner@andrew.cmu.edu

William Wagner, Ph.D.
McGowan Institute for Regenerative Medicine
University of Pittsburgh
T: 412-235-5138
wagnerwr@upmc.edu

Herbert Waite, Ph.D.
Marine Science Institute
University of California, Santa Barbara
T: 805-893-2817
waite@lifesci.ucsb.edu

Johnathan Wall, Ph.D.
Human Immunology & Cancer Program
University of Tennessee Graduate
School of Medicine
T: 865-544-9165
jwall@mc.utmck.edu

Lihong Wang, Ph.D.
Optical Imaging Laboratory
Texas A&M University
T: 979-847-9040
LWang@tamu.edu

Stephan Weber, Ph.D.
Department of Biomedical Engineering
The Cleveland Clinic Foundation
T: 216-445-3277
webers@bme.ri.ccf.org

Richard Weir, Ph.D.
Department of Physical Medicine &
Rehabilitation
Northwestern University Feinberg
School of Medicine
T: 312-238-6500
rweir@northwestern.edu

Shimon Weiss, D.Sc.
Department of Chemistry and
Biochemistry
University of California, L.A.
T: 310-794-0093
sweiss@chem.ucla.edu

Jack Werner, Ph.D.
Visual Psychophysics
University of California Davis
jswerner@ucdavis.edu

Dwayne Westenskow, Ph.D.
Department of Anesthesiology
University of Utah
T: 801-581-2478
dwayne.westenskow@hsc.utah.edu

Stephen White, Ph.D.
Department of Physiology & Biophysics
University of California at Irvine
T: 949-824-7122
blanco@helium.biomol.uci.edu

David Williams, Ph.D.
Center for Visual Science
University of Rochester
T: 585-275-8672
david@cvs.rochester.edu

Karl Dane Wittrup, Ph.D.
Chemical Engineering Department &
Division of Biological Engineering
Massachusetts Institute of Technology
T: 617-253-4578
wittrup@mit.edu

Jonathan Wolpaw, M.D.
Laboratory of Nervous System Disorders
Wadsworth Center
New York State Department of Health
and SUNY
T: 518-473-3631
wolpaw@wadsworth.org

John Wong, Ph.D.
Department of Radiation Oncology
William Beaumont Hospital
T: 248-551-6583
jwong@beaumont.edu

Ajit Yoganathan, Ph.D.
School of Biomedical Engineering
Georgia Institute of Technology
404-894-2849
ajit.yoganathan@bme.gatech.edu

Yan Yu, Ph.D.
Department of Radiation Oncology
University of Rochester Medical Center
T: 585-275-9988
yan_yu@URMC.Rochester.edu

NIH Bioengineering Consortium (BECON)

CHAIR

Cancer Institute (NCI)
Daniel C. Sullivan, M.D.
Associate Director
Diagnostic Imaging Program
National Cancer Institute, NIH
Executive Plaza North, Room 800
6130 Executive Boulevard
Bethesda, MD 20892-7440
Phone: 301-496-9531
Fax: 301-480-5785
ds274k@nih.gov

MEMBERS

Aging Institute (NIA)
Winifred K. Rossi, M.A.
Health Program Specialist
Genetic Epidemiology and Translational Research Geriatrics Program
National Institute on Aging, NIH
7201 Wisconsin Avenue, Suite 3E-327
Bethesda, MD 20892-9205
Phone: 301-496-3836
Fax: 301-402-1784
wr33a@nih.gov

Alcohol Abuse and Alcoholism (NIAAA)
Karen Peterson, Ph.D.
Research Policy and Special Programs
Office of Scientific Affairs
National Institute on Alcohol Abuse and Alcoholism, NIH
5635 Fishers Lane, Room 2017
Rockville, MD 20892
Phone: 301-451-3883
Fax: 301-480-2358
kpeterso@mail.nih.gov

Allergy and Infectious Diseases (NIAID)
Richard Morris, M.S.E, Ph.D.
Special Expert, Immunology and Transplantation (DAIT)
National Institute of Allergy and Infectious Diseases, NIH
6700-B Rockledge Drive, Room 5162
Bethesda, MD 20892
Phone: 301-594-7634
Fax: 301-402-2571
rm69e@nih.gov

Allergy and Infectious Diseases (NIAID)
Gregory Milman, Ph.D.
Director
Office of Innovation and Special Programs
National Institute of Allergy and Infectious Diseases, NIH
6700-B Rockledge Drive, Room 2140
Rockville, MD 20814-7610
Phone: 301-496-8666
Fax: 301-402-0369
gmilman@niaid.nih.gov

Allergy and Infectious Diseases (NIAID)
Maria Giovanni, Ph.D.
Assistant Director for Microbial Disease
Division of Microbiology and Infectious Disease
National Institute of Allergy and Infectious Diseases, NIH
6700-B Rockledge Drive, MSC-7630
Bethesda, MD 20892
Phone: 301-496-1884
Fax: 301-402-0892
Mg37u@nih.gov

Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
James Panagis, M.D., M.P.H.
Director
Orthopaedics Program
National Institute of Arthritis and Musculoskeletal & Skin Diseases, NIH
45 Center Drive, Room 5AS-37K
Bethesda, MD 20892-6500
Phone: 301-594-3513
Fax: 301-480-4543
jp149d@nih.gov

Biomedical Imaging and Bioengineering (NIBIB)
Christine Kelley, Ph.D.
Director
Division of Applied Science and Technology
National Institute of Biomedical Imaging and Bioengineering, NIH
6707 Democracy Boulevard, Suite 200
Bethesda, MD 20892
Phone: 301-451-4772
Fax: 301-480-4973
kelleyc@mail.nih.gov

Cancer Institute (NCI)
Edward Monachino, M.S.E.E.
Senior Technology Manager
National Cancer Institute, NIH
Bldg 31, Room 10A49, MSC 2580,
Bethesda, MD 20892-2580
Phone: 301-496-1550
monachie@mail.nih.gov

Child Health and Human Development (NICHD)
Louis A. Quatrano, Ph.D.
Chief
Applied Rehabilitation Medicine Branch
National Institute of Child Health and Human Development, NIH
Executive Plaza South, Room 2A03
6120 Executive Boulevard
Bethesda, MD 20892
Phone: 301-402-2242
Fax: 301-402-0832
lq2n@nih.gov

Child Health and Human Development (NICHD)
Michael Weinrich, M.D.
Director
National Center for Medical Rehabilitation Research
National Institute of Child Health and Human Development, NIH
6100 Executive Boulevard, Room 8B07
Bethesda, MD 20852
Phone: 301-402-2242
Fax: 301-402-0832
weinricm@mail.nih.gov

Deafness and Other Communication Disorders (NIDCD)
Nancy Freeman, Ph.D.
Scientific Program Director
National Institute on Deafness and Other Communication Disorders, NIH
6120 Executive Boulevard, Suite 400-C
Bethesda, MD 20892-7180
Phone: 301-435-0597
Fax: 301-402-6251
freemann@mail.nih.gov

Dental and Craniofacial Research (NIDCR)
Eleni Kousvelari, D.D.S., D.Sc.
Chief
Biomaterials, Biomimetics, and Tissue Engineering Branch
National Institute of Dental and Craniofacial Research, NIH
45 Center Drive, Room 4AN-18A
Bethesda, MD 20892
Phone: 301-594-2427
Fax: 301-480-8318
kousvelari@de45.nidr.nih.gov

Diabetes and Digestive and Kidney Diseases (NIDDK)
Maren R. Laughlin, Ph.D.
Director
Metabolism Program
National Institute of Diabetes and Digestive & Kidney Diseases, NIH
6707 Democracy Boulevard, Room 6101
Bethesda, MD 20892-5460
Phone: 301-594-8802
Fax: 301-480-3503
Maren.laughlin@nih.gov

Drug Abuse (NIDA)
Thomas G. Aigner, Ph.D.
Health Scientist Administrator
Division of Neuroscience and Behavioral Research
National Institute on Drug Abuse, NIH
Neuroscience Center, Room 4282
6001 Executive Boulevard
Bethesda, MD 20892-9555
Phone: 301-443-6975
Fax: 301-594-6043
ta17r@nih.gov

Environmental Health Sciences (NIEHS)
William A. Suk, Ph.D., M.P.H.
Deputy Director for Program Development
Division of Extramural Research and Training
National Institute of Environmental Health Sciences, NIH
P.O. Box 12233
111 Alexander Drive, MD EC-27
Research Triangle Park, NC 27709
Phone: 919-541-0797
Fax: 919-541-4937
ws22e@nih.gov

Eye Institute (NEI)
Richard S. Fisher, Ph.D.
Division of Extramural Research
National Eye Institute, NIH
Executive Plaza South, Suite 350
6120 Executive Blvd, MSC 7164
Bethesda, MD 20892-7164
Phone: 301-451-2020
Fax: 301-402-0528
rf75s@nih.gov

General Medical Sciences (NIGMS)
Warren Jones, Ph.D.
Chief
Biochemistry and Bio-Related Chemistry Branch
Pharmacology, Physiology, and Biological Chemical Division
National Institute of General Medical Sciences, NIH
Building 45, Room 2AS-43
45 Center Drive
Bethesda, MD 20892-6200
Phone: 301-594-5938
Fax: 301-480-2802
jonesw@nigms.nih.gov

Heart, Lung, and Blood Institute (NHLBI)
Martha Lundberg, Ph.D.
Program Director
Clinical and Molecular Medicine
National Heart, Lung, and Blood Institute, NIH
6701 Rockledge Drive, Room 9146
Bethesda, MD 20892
Phone: 301-435-0513
Fax: 301-480-1335
LundberM@nhlbi.nih.gov

Human Genome Research Institute (NHGRI)
Jeffery A. Schloss, Ph.D.
Program Director
Technology Development Coordination
National Human Genome Research Institute, NIH
5635 Fishers Lane, Suite 4076
Bethesda, MD 20892-9305
Phone: 301-496-7531
Fax: 301-480-2770
jeff_schloss@nih.gov

National Library of Medicine (NLM)
Valerie Florance, Ph.D.
Program Officer
Division of Extramural Programs
National Library of Medicine, NLM
6705 Rockledge Drive, Room 301
Bethesda, MD 20892
Phone: 301-594-4882
Fax: 301-402-2952
floranv@mail.nlm.nih.gov

Mental Health (NIMH)
Michael F. Huerta, Ph.D.
Associate Director
Division of Neuroscience and Basic Behavioral Science
National Institute of Mental Health, NIH
Neuroscience Center, Room 7202
6001 Executive Boulevard
Bethesda, MD 20892-9645
Phone: 301-443-3563
Fax: 301-443-1731
mh38f@nih.gov

Neurological Disorders and Stroke (NINDS)
Joseph Pancrazio, PhD
Program Director
Extramural Research Programroscience
National Institute of Neurological Disorders and Stroke, NIH
Neuroscience Center, Room 2205
6001 Executive Blvd MSC 9525
Bethesda, MD 20892
Phone: 301-496-1447
Fax: 301-480-1080
jp439m@nih.gov

Research Resources (NCRR)
Michael T. Marron, Ph.D.
Associate Director for Biomedical Technology
National Center for Research Resources, NIH
6705 Rockledge Drive, Room 6160
Bethesda, MD 20892-7965
Phone: 301-435-0753
Fax: 301-480-3659
marron@nih.gov

Center for Scientific Review (CSR)
Eileen Bradley, D.Sc.
Scientific Review Administrator
Diagnostic Imaging Review Group
Center for Scientific Review, NIH
6701 Rockledge Drive, Room 5120
Bethesda, MD 20892
Phone: 301-435-1179
Fax: 301-480-2241
eb15y@nih.gov

NIH Clinical Center (CC)
Alexander Gorbach, Ph.D.
Staff Scientist
Diagnostic Radiology Department
Clinical Center, NIH
Building 10, Room 1C-660
10 Center Drive, MSC 1182
Bethesda, MD 20892
Phone: 301-496-1489
Fax: 301-402-0380
gorbach@ninds.nih.gov

Office of Intramural Research (OIR)
Philip S. Chen, Jr., Ph.D.
Senior Advisor to the Deputy Director for Intramural Research
Office of the Director, NIH
Building 1, Room 140
1 Center Drive
Bethesda, MD 20892-0152
Phone: 301-496-3561
Fax: 301-402-0027
pc17w@nih.gov

Office of Research Services (ORS)
Richard D. Leapman, Ph.D.
Chief
Supramolecular Structure and Function Resource
Bioengineering and Physical Science Program
Office of Research Services, NIH
Building 13, Room 3N17
9000 Rockville Pike
Bethesda, MD 20892
Phone: 301-496-2599
Fax: 301-496-6608
rl12b@nih.gov

Department of Energy (DOE)
Michael V. Viola, M.D.
Director
Medical Sciences Division
U.S. Department of Energy
19901 Germantown Road, Room J-115
Germantown, MD 20874-1290
Phone: 301-903-5346
Fax: 301-903-0567
michaelv@science.doe.gov

National Science Foundation (NSF)
Bruce Hamilton, PhD
Director
Division of Bioengineering & Environmental Systems
National Science Foundation
4201 Wilson Boulevard, Room 1205
Arlington, VA 22230
Phone: 703- 292-7066
Fax: 703- 292-9098
bhamilto@nsf.gov

National Institute of Standards and Technology (NIST)
Mrunal Chapekar, Ph.D.
Program Manager
National Institute of Standards and Technology
100 Bureau Drive - MS 4730
Gaithersburg, MD 20899
Phone: 301-975-6846
Fax: 301-869-1319
mrunal.chapekar@nist.gov

EXECUTIVE SECRETARY

Biomedical Imaging and Bioengineering (NIBIB)
Richard Swaja, PhD
Senior Science Advisor
National Institute of Biomedical Imaging and Bioengineering, NIH
6707 Democracy Boulevard, Suite 200
Bethesda, MD 20892
Phone: 301-451-4779
Fax: 301-480-4973
Email: swajar@nibib.nih.gov

Biomedical Imaging and Bioengineering (NIBIB)
Mariaileen Sourwine, MS
Biomedical Engineer
National Institute of Biomedical Imaging and Bioengineering, NIH
6707 Democracy Boulevard, Suite 200
Bethesda, MD 20892
Phone: 301-451-4775
Fax: 301-480-4973
Email: sourwine@nibib.nih.gov

Grantee Summary Reports

PI: Robert H. Bartlett, M.D.
University of Michigan
Department of Surgery
2920 Taubman Center
1500 E. Medical Center Drive
Ann Arbor, MI 48109-0331
T: 734-936-5822
F: 734-936-5830
robbar@umich.edu

PROJECT TITLE: Implantable Total Artificial Lung

PARTNERS' NAMES AND AFFILIATIONS:

James Grotberg, Ph.D., M.D. (Biomedical Engineering Department, University of Michigan)
Joseph Bull, Ph.D. (Biomedical Engineering Department, University of Michigan)

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

ABSTRACT

Severe chronic lung disease is a common cause of death in the United States. The only successful treatment is lung transplantation, however the supply of lung donors is limited because many organ donor subjects have poor lung function. As a consequence most patients on the waiting list for lung transplant die before a donor organ becomes available. Patients awaiting kidney or heart transplant can be supported by mechanical devices (dialysis, LVAD) for months or years until a suitable organ donor becomes available. The only mechanical support system for respiratory failure is extracorporeal life support commonly known as ECMO, which can be used safely for only a few weeks. Consequently there is a need for a mechanical device to replace lung gas exchange function for a period of months for patients with severe chronic lung disease awaiting lung transplantation. The purpose of this project is to design, develop, test and bring to prototype stage a totally implantable artificial lung.

Our group had extensive preliminary experience with the development of an artificial lung leading up to the BRP grant. We had identified the problems as: design of a very low resistance high efficiency membrane lung, attachment to the pulmonary artery and distal pulmonary artery or left atrium, evaluating right ventricular performance and optimizing lung design so that the lung could be perfused completely by the right ventricle, and addressing the problems of thrombosis and embolization. The bio-engineering component includes evaluation of factors leading to resistance and impedance to blood flow, factors needed to optimize gas exchange, resistance and impedance related to conduit connectors, and mathematical modeling of blood flow as related to gas exchange and thrombosis. Fabrication of prototype devices is subcontracted to MC3 a local Ann Arbor bio-engineering R&D company.

STATUS OF RESEARCH AND PARTNERSHIP

The mathematical modeling of blood flow through the test device has been a major focus of the bio-engineering team. Because flow is driven by the right ventricle it is pulsatile flow at relatively low pressure. The detailed analysis of secondary flows generated by pulsatile flow around a series of parallel fibers had not been evaluated in the past. This created a fascinating mathematical modeling project which is nearly complete. This will allow us to position the fibers and the distances between the fibers in an optimal fashion in order to optimize gas exchange. Mathematical modeling also allows us to minimize areas of low flow which would lead to thrombosis. Dr. Joe Bull has been leading the mathematical modeling project. Device design and prototype manufacture at MC3 has proceeded throughout the project. A very low blood flow resistance high efficiency gas exchange device has been created in prototype and tested in several configurations. This device is trademarked as "Bio Lung." The Bio Lung

has a very low blood flow resistance, excellent gas exchange, and is relatively easy to manufacture. The basic design is a blood outside hollow fiber capillary device in which blood flows to the center of the hollow fiber bundle and flows out to exit ports on the outside of the bundle. Inlet/outlet connectors are designed for rapid attachment. The configuration has been modified several times to an ideal oval shape which will be used in a paracorporeal fashion and is evaluated in that fashion in experimental sheep. To date the hollow fibers have been made of microporous polypropylene which can cause problems in plasma leakage from the blood to the gas side and also in gas leakage from the gas to the blood side. These problems are minimal in the sheep but will be major problems in human application because of lipids absorbing to the membranes causing plasma leakage. The microporous fibers have been easy to work with for prototype development, but a major effort in the current year has been to acquire hollow fibers with a solid membrane to avoid the leakage problems. Our negotiations with the Membrana from Germany have allowed us to acquire fiber mats of near-solid PMP membrane which are currently undergoing testing. In addition contacts with two companies in Japan are underway to evaluate silicone rubber fibers and solid PMP fibers.

The physiology of right ventricular function has been a major part of our evaluation. The combination of mathematical modeling and direct experimental evidence has identified several problems which we have solved. The problem is that although resistance to flow through the Bio Lung is low the impedance is relatively high, specifically the first and second harmonic of impedance are significantly higher than the normal lung and this is the major factor leading to right ventricular failure. In the past year we evaluated a compliance chamber designed to solve this problem. The compliance chamber was evaluated in bench in vitro studies and in live animal studies. We identified that a compliance of .5cc per mm mercury pressure dampens the first harmonic to safe levels. Compliance of 5ml per mm mercury pressure eliminates the first and second harmonic problem and minimizes pulmonary regurgitation. The ideal compliance chamber will have a compliance of 1-2cc per centimeter water pressure and the current prototypes include this modification. Problems of attachment of the membrane lung to the patient were addressed in the past year using sheep experiments. Our experiments have focused on placing the implantable lung from the proximal pulmonary artery to the left atrium. Parallel experiments going on elsewhere are studying proximal pulmonary artery to distal pulmonary artery attachment. There are advantages and disadvantages to each. Our current experience indicates that pulmonary artery to left atrium placement will be feasible. Managing the implantable artificial lung without anticoagulation is a major goal. Our laboratory has focused on plastic surfaces which release nitric oxide into the blood immediately adjacent to the membrane. This prevents platelet adhesion to the surface and is the most effective non-thrombogenic surface we have evaluated. During the past year we evaluated the possibility of including low levels of nitric oxide gas into the ventilating gas to minimize platelet adhesion on the membranes which are more difficult to coat with NO releasing plastic. Also in the past year we have completed the development of a complex chronic pulmonary hypertension model in the sheep specifically to evaluate the effect of the implantable lung on chronic pulmonary hypertension, simulating the human disease of primary pulmonary hypertension. This model includes intermittent injection of Sephadex beads into the pulmonary circulation. We have determined that a dose of 0.5 grams of beads every other day for 60 days results in a chronic stable evaluable model of pulmonary hypertension. With all of these problems addressed during the next year we plan to evaluate chronic (7-30 day) paracorporeal implantation of the artificial lung into a series of sheep to evaluate factors which might limit long-range effectiveness such as thrombosis gas exchange and infection.

ISSUES

Regular conferences and communication between the members of the partnership have been essential for progress. Many undergraduate, graduate medical and bio-engineering students are participating in this project. The research is proceeding with few limitations. The major factor limiting the chronic animal experiments is the use of non-porous hollow fibers in design of the implantable lung. These experiments are planned for the coming year.

PI: Lance B. Becker, MD
University of Chicago
Department of Medicine
5841 S. Maryland Avenue
MC5068
Chicago, IL 60637
T: 773 702 9500
F: 773 702 3135
lbecker@medicine.bsd.uchicago.edu
http://errc.bsd.uchicago.edu

PROJECT TITLE: "Optimizing Heart and Brain Cooling during Cardiac Arrest"

PARTNERS' NAMES AND AFFILIATIONS:

Terry L. Vanden Hoek, MD (University of Chicago, Dept of Medicine)
Craig Wardrip, DVM (University of Chicago, Department of Surgery)
Michael F. O'Connor, MD (University of Chicago, Department of Anesthesia)
Danhong Zhao, MD (University of Chicago, Department of Medicine)
Travis Anderson (University of Chicago, Department of Medicine)
Kenneth E. Kasza, PhD (Argonne National Laboratory)
John Oras, PhD (Argonne National Laboratory)
Jeffrey Franklin, PhD (Argonne National Laboratory)
Tai-Hsin Chien, PhD (Argonne National Laboratory)
David Beiser, MD (University of Chicago, Dept of Medicine)

ABSTRACT

Cardiac arrest currently has a less than 5% survival rate and hypothermia may be an important therapy to improve this poor outcome. The poor survival is due to the brief time paramedics have to restart blood flow to ischemia-sensitive organs such as the heart and brain. The recent NIH PULSE Conference identified cooling as one of the "most promising" areas of research to improve cardiac arrest survival. While techniques such as cardiopulmonary bypass and aortic flush can rapidly induce hypothermia, the complexity of their initial setup makes them less practical for cooling in the field. Therefore, designing a rapid cooling system usable in the field is an ideal focus for a bioengineering research partnership in which engineers and clinicians combine efforts. We hypothesize that cooling after cardiac arrest can be rapid (despite the low blood flow conditions of CPR) and practical for paramedics under field conditions, and will improve survival. We propose a novel cooling system to be developed in a swine model of cardiac arrest, with the ultimate goal of human application by paramedics. Our partnership of physicians, biologists, and engineers at the University of Chicago and Argonne National Laboratory has modified for medical application ice slurry technology originally developed for cooling large buildings. These microparticle ice slurries flow like liquids, but have up to 8 times the cooling capacity of a similar volume of cold liquid (0-4C) without ice, facilitating heat transfer with lower volumes and flow. For this project, we have developed two novel ice-particle slurries: (a) a saline-based ice slurry (saline slurry) that can be used through existing intravenous catheters or pumped into the stomach and (b) a perfluorocarbon-based ice slurry (PFC slurry) for endotracheal instillation into the lungs. In preliminary studies, these prototype ice slurries used in tandem have achieved remarkable cooling rates of approximately 0.5°C/min within the heart and brain during cardiac arrest with chest compressions – and have the potential to cool even faster with improved formulations. Using prototype PFC slurry as a coolant in the lungs of normal animals, some toxicity resulted (consistent with other reports of PFC toxicity), but animals survived unassisted for 48 hours with normal oxygenation with improving respiratory function, and had only mild pathological changes on histology. Thus our preliminary data suggests developing an optimal cooling method with minimal adverse effects to be a realistic goal. This hypothermia system represents a new option for

induction of intra-arrest low flow hypothermia to be rapidly performed by paramedics in the field. To further promote the development of this project, a multi-center international advisory board of noted cardiac arrest experts will assist the Partnership. With a cooling system engineered to surmount this heat-transfer challenge, multi-center animal studies could quickly lay the scientific foundation for implementing what could become a new and effective cardiac arrest treatment by paramedics in the field. The development of cooling therapies may also be useful in the treatment of myocardial infarction, stroke, brain injury, as well as in comatose survivors of cardiac arrest.

STATUS OF RESEARCH AND PARTNERSHIP

1. We have developed an onsite calorimetric technique to quantify more precisely the quality of our slurry in terms of cooling content. This technique has the advantage that it can be used onsite to verify that the slurry administered to each animal is within expected limits of ice-content. This new capability allows us to confirm the percentage of ice content is within a 3% margin of error for any slurry administered to an animal. 2. We have performed a series of experiments to validate our use of fluorescent microspheres to estimate regional blood flow in multiple organs during cardiac arrest. Determining blood flow to vital organs (heart, brain, etc) during cardiac arrest is known to be very difficult and in our application we proposed using microspheres to determine blood flow during cardiac arrest. 3. We have further optimized an improved saline-ice slurry that has higher ice-content for experimental use. Using the calorimeter (described in #1 above), we determined that the majority of our prior experiments were conducted using slurries with ice-contents between 18 and 25%. In the laboratory we had prepared slurry with ice-contents approaching 50%, but due to the heat gain from ambient sources, smoothing to achieve finishing there has been significant loss of ice content over time – we discovered the actual slurry we were delivering to each animal was much lower than we originally believed (i.e. 18-25%). With continued development of our "in-laboratory" onsite slurry manufacturing methods we have now advanced this to nearly 40% ice-content (ranges from 37% to 42% in our latest efforts). This improved slurry maintains good flowablitiy. 4. We have conducted additional studies directly comparing the cooling effects of our ice slurry (20% ice content) versus cold saline when equal volumes are administered by an intravenous route. We hypothesized that an intravenous bolus of slurry would induce mild hypothermia (32 – 34 ºC) more quickly than an equal bolus of chilled saline. Intravenous bolus (50 ml/kg) of slurry or chilled 1.5% normal saline were administered while cerebral cortex, tympanic membrane, inferior vena cava and rectal temperatures, heart rate and blood pressure were recorded for 1 hour. Compared to sedated controls, core brain temperatures of the saline and slurry groups dropped quickly at rates of -11.6 ± 1.8 ºC/h and -18.2 ± 2.9 ºC/h respectively during the first 20 minutes, with the greatest absolute temperature drop measured 3.4 ± 0.4 ºC versus 5.3 ± 0.7 ºC ($p = 0.009$). None of the saline group reached $T \leq 34$ºC but all of the slurry pigs achieved core temperatures below 34 ºC and maintained target cooling for hours following the bolus. No adverse physiologic events were observed. We concluded that cold intravenous fluids can rapidly induce hypothermia in pigs with intact circulation. A two-phase (liquid plus ice) saline slurry cools more rapidly and decreases the fluid bolus volume necessary to achieve hypothermia. This work was presented at the Wolf Creek Resuscitation Conference and has been accepted for publication in *Critical Care Medicine*. 5. We are currently engaged in a series of studies to determine the effectiveness and possible adverse effects of the GI route for cooling with ice-slurry. To begin to optimize this method of slurry administration we have modified standard gastric tubes to reduce plugging and have added a thermocouple temperature probe to the end of the tube. We have performed studies on cooling effects using 10ml/kg, 20ml/kg, and 30ml/kg of ice slurry (with 35-40% ice content). 6. We have developed a preliminary mathematical model for estimation the amount of slurry required to achieve different levels of cooling using ice-slurry. We have extended the work from prior studies that have sought to understand the mechanisms of human thermoregulation through theoretical models and experimental measures.
7. We have performed pilot experiments to determine the effect of intra peritoneal delivery of ice-slurry for cooling.

PI: Michael W. Berns
Department of Biomedical Engineering
University of California, Irvine
1002 Health Sciences Rd East
Irvine, CA 92612
T: (949) 824-6291
F: (949) 824-8413

PROJECT TITLE: Integrated Platform for Chemical Analysis of Live cells

PARTNERS' NAMES AND AFFILIATIONS: Michael W. Berns, Beckman Laser Institute and Department of Biomedical Engineering; Nancy Allbritton, Department of Physiology and Biophysics and Biomedical Engineering; Chris Sims, Department of Biophysics and Physiology; G.P. Li, Department of Electrical and Computer Engineering; and Vasan Venugopalan Beckman Laser Institute and the Department of Material Science and Chemical Engineering.

NIH GRANTING INSTITUTE/CENTER: NIBIB

ABSTRACT
The overall aim of this partnership was to design, build and test an integrated optical and microfluidics system that will enable the performance of novel biochemical assays in single, living cells. The development process involves basic studies of the physical mechanisms of laser interactions with cells and polymeric materials used to manufacture the chips, basic engineering of polymer-based microfluidic devices, integration of the microfluidic devices and microscope platform, and further development of novel biochemical assays to be performed with the integrated system. A final practical goal was either to license this technology to a well-established company or spin off a company to commercialize it.

STATUS OF RESEARCH AND PARTNERSHIP
During the four years of this project significant progress has been made in areas of laser interactions with cells, MEMS integration for on-chip cell lysis, and instrument control. Additionally a company was spun-off involving two of the investigators to commercialize technology that was suggested in the original application.

An important focus has been to develop a system to visualize the dynamics of laser microbeam interactions with cells when applied for cell lysis and optoporation. These dynamics are critical to understanding the temperatures and pressures attained and the hydrodynamics involved in these processes and their implications for developing a MEMS device using this technology. The imaging system we have developed allows us to do time-resolved imaging on cell samples from sub-nanosecond to 100's of microseconds with a spatial resolution of a few microns using a Nd:YAG laser. Studying the dynamics has allowed us to calculate shock wave speeds and peak pressures and measure cavitation bubble sizes, oscillation times and bubble energies and time-scales of the process. Current work concentrates on producing a physical model of the breakdown process which would allow us to correlate the physical and biological effects. Additional efforts include the study of micro-channel and micro-cavity designs to optimize the lysis of cells and subsequent removal of lysate in polymer chips. Computer imaging technology and robotic control instrumentation has advanced such that a laser microscope can be controlled from a computer terminal over an internet connection.

We have developed strategies using ultraviolet light to polymerize mixed monomer solutions onto the surface of a poly(dimethylsiloxane) (PDMS) microfluidic devices. By including monomers with different chemical properties, electrophoretic separations were optimized for a test set of analytes. The properties of surfaces grafted with a single neutral monomer, a neutral and a negative monomer, or a neutral,

negative, and cross-linking monomer were assessed. This has helped to solve one of the most limiting problems in polymer microfluidic chips—control of the wall surface chemistry. High quality separations were achieved in PDMS microfluidic channels with cross-linked coatings. The separation efficiency for biologically relevant peptides (kinase substrates) on these surfaces was as high as 18,600 theoretical plates in a 2.5 cm channel, and separations between two different peptides occurred in as little as 400 ms after injection. The simultaneous separation of five kinase and phosphatase substrates was also demonstrated. By carefully selecting mixtures of monomers with the appropriate properties, it may be possible to tailor the surface of PDMS for a large number of different electrophoretic separations. Biochemical assays continue to be developed for use in the microfluidic chips. Fluorescent labeling and deprotection protocols for the preparation of enzyme substrates to be used in the biochemical assays are being optimized and put into practice.

Finally, the commercialization of the single cell analysis system has progressed both through the filing of new UC intellectual property disclosures, provisional patent applications, and licensing of earlier patented technology to a new start-up company. The chemical surface treatment has been demonstrated to be of great utility to polymer microfluidic chips as well as to other biomedical applications such as implantable devices. UC is currently in negotiation with two companies to license relevant portions of the surface grafting technology.

ISSUES

None. We've achieved most of our goals, and a company was spun-out to commercialize a related product.

PI: John M. Boone, Ph.D.
Professor of Radiology and
Professor of Biomedical Engineering
Department of Radiology
UC Davis Medical Center
4701 X Street, Imaging Research Center
Sacramento, California 95817
916-734-3158
jmboone@ucdavis.edu

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, Stanford University, Hanuman University (Philadelphia, PA), University of Arizona, Tucson.

GRANTING NIH INSTITUTE/CENTER: NIBIB, previously 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 image the breast alone on a live women using a clinical CT scanner. Therefore, in this Bioengineering Research Partnership proposal, we have teamed with scientists from around the country to design, build, and test a CT scanner designed to image the breast. A team comprised of medical physicists, physicians, mechanical and electrical engineers, and breast cancer advocates will collaborate on the design of the breast CT scanner. Cone beam flat panel technology will be used to produce a scanner capable of 10 second breast scanning, and the scanner development will also include a breast immobilization system (acrylic cylinders), a breast CT table, a fast reconstruction computer, and a computer workstation customized for efficient viewing breast CT images.

The scanner will be built, tested, and optimized at UC Davis over a period of 3 years involving 9 specific aims. After the breast CT scanner is tested in a brief phase I trial (2 specific aims), it will be moved to the breast imaging clinic for a phase II trial where approximately 120 women will be imaged (4 specific aims). This phase II trial will evaluate the efficacy of breast CT for the early detection of breast cancer in a group of women likely to have breast cancer (BIRADS 4 & 5). Additionally, the breast image data will be studied for its utility in automating the analysis of the normal breast architecture, and for computerized cancer detection. In year 5 of the proposed research, two specific aims utilize the breast CT data and corresponding mammography images (on ~240 breasts) to evaluate the ideal observer performance and human (mammographer) detection performance attributes of the breast CT scanner.
At the end of the proposed research involving 17 specific aims, the potential of breast CT will have been evaluated both qualitatively and quantitatively. A tested, high quality prototype breast CT scanner would be ready to be enlisted in a phase III trial (beyond the scope of this proposed research), if further testing is

warranted. Performance data acquired in the present study would allow the proper design (power, etc.) of a phase III trial. If breast CT lives up to its enormous potential based on initial imaging, breast cancer would be detectable far before 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 PARTNERSHIP AND RESEARCH

The BRP partnership is proceeding with what we view as excellent progress. Although the fabrication of our prototype breast CT scanner is behind schedule by almost a year, we have pursued a significant number of problems and scientific issues associated with the breast CT project using an early prototype system in the PI's laboratory. The information gleaned from these experiments has been crucial in the design of the more advanced prototype breast CT scanner to be constructed under the auspices of this BRP funding. The PI has met with the co-PIs at partner institutions (particularly those in California) on numerous occasions. We are planning a meeting of all consultants associated with this project in September. The consultants will form what is in effect an external advisory board, and we will conduct this meeting much like a site visit. We have ordered and have received the large components associated with the fabrication of the prototype breast CT scanner, including the x-ray tube and x-ray generator system, and the large motor which will serve to rotate the moving gantry of the system. The x-ray detector is slated to be received in September 2004. We anticipate having a working second prototype CT scanner by summer 2005.

ISSUES

We feel that we have made substantial progress towards the development of breast CT, and have studied numerous scientific underpinnings that will help us direct the design of the physical system. 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 probably not in the loop as much as they should be. We are striving to improve this situation over the next year.

PI: Michael Bottlang, PH.D.
Legacy Emanuel Hospital and Health Center
Legacy Clinical Research & Technology Center
1225 NE 2nd Ave
Portland, Oregon 97215
Phone: 503 413 5457
Fax: 503 413 4942
mbottlan@lhs.org
http://www.legacyhealth.org/findus/otherlocations/techcenter.ssi

PROJECT TITLE: An organotypic model of traumatic brain injury

PARTNERS' NAMES AND AFFILIATIONS:
Dr. Simon (Dow Neurological Institute), Dr. Xiong and Dr. Lusardi (Dow Neurological Institute), Dr. Rochefort (Oregon State University)

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

ABSTRACT
The past decade has witnessed intense scientific activity to investigate molecular mechanisms of traumatic brain injury, driven by overwhelming evidence that neuroprotection by pharmacological inhibition of apoptosis has the potential to dramatically reduce the effects of brain trauma. Key requisite for the systematic investigation of neuroprotective agents is an accurately characterized, clinically relevant in vitro brain injury model. Despite this obvious need, the ability to deliver such defined, realistic trauma to specimens in vitro lags far behind the sophistication of molecular and biochemical assays used to measure the response. This Bioengineering Research Partnership brings together neurobiologists and bioengineering scientists to developed an *in vitro* brain injury model, which subjects organotypic brain cultures to acceleration-induced shear injury. In this model, organotypic brain cultures realistically model the *in vivo* apparent heterogeneous cell population in a three-dimensional cellular matrix, while inertial acceleration-induced shear strain delivers a scalable, defined, and clinically relevant mechanical insult. We hypothesized that our acceleration model of organotypic brain cultures can realistically reproduce traumatic brain injury, in which the delivered shear strain magnitude can be quantified on a cellular level. Exercising our model, we determine cell type specific injury vulnerability and biological injury cascades in response to a defined mechanical insult.
To date, we complete a formal experimental characterization of our novel brain injury system, including assessment of the delivered angular acceleration magnitude and determination of the constitutive properties of the organotypic culture (Aim 1). The resulting experimental source data were required to formulate a validated analytical model that allows computational simulation of the shear injury throughout the brain specimen for any point in time during the primary mechanical insult (Aim 2). Subsequent to this rigorous system characterization, we exercised the brain injury model to establish dose/response histories (Aim 3). Over the past year, we implemented the *in vitro* brain injury system to investigate cell-type specific injury susceptibility, time-history of the biological injury cascade, and effects of hypoxic brain injury secondary to the mechanical insult (Aim 4). We furthermore advanced the existing model toward a 3rd-generation *in vitro* brain injury system, which will be more user-friendly, robust, and allows for higher through-put experiments in six-well culture plates.
Upon successful completion, the results of this integrative research approach will yield a well-characterized, scalable, reproducible and clinically relevant brain injury model. Considering the vast interest in therapeutic interventions now under development aimed at inhibiting the cascade of secondary effects of primarily mechanical brain injuries, our organotypic trauma model will directly address the

rapidly increasing demand for a well characterized, experimental system to deliver a clinically relevant traumatic insult.

STATUS OF RESEARCH AND PARTNERSHIP

In the second year of our research partnership, we were able to employ the brain injury model for characterization of brain injury mechanisms, and we further optimized the brain injury model itself.

Model Employment: Cell-type specific injury thresholds were assessed with quantitative image analysis of confocal microscopy images after neuron-specific straining of organotypic cultures with Neu-N and nuclei staining with DAPI. The time-history of cell death following shear injury was quantified by means of lactate dehydrogenase up to 56 hours post-injury. Next to cell death, sub-lethal cell injury was explored by inclusion of additional outcome indicators, including transcription factors cFos and heat shock proteins as indicators of the cellular stress state, and intracellular calcium to indicate whether homeostasis has been disrupted and/or neuronal sensitivity to agonist has been altered.

Model Advancement: The brain injury model was adapted for use with hippocampal slices in addition to whole brain slices. Hippocampal slices can be maintained far easier and more reliable as compared to entire brain slices and are routinely used for the study of synaptic transmission and plasticity (e.g. long-term potentiation), an analysis of neuronal network and its function. This adaptation was crucial to advance the model into a robust tool for potential use by a range of researchers investigating traumatic brain injury (TBI) in vitro. It further takes advantage of increased anatomic and functional uniformity from slice to slice, facilitating analysis of hippocampal regions CA1-CA3 and the dentate gyrus under confocal microscopy. Recognizing the potential of this easy-to-maintain TBI model, we currently design a 3rd generation TBI model, which allows for simultaneous injury to cultures in a standard six-well culture plate with six individual Millicell inserts. In place of a relatively large mechanical pendulum, acceleration will be controlled by a pneumatic actuator. This design is geared toward an easy-to-maintain and cost-effective device for commercialization of an in vitro injury simulator. This system would hold considerable attraction for researchers in need of high-throughput investigation of TBI in vitro.

ISSUES

The collaboration within the BRP has progressed smoothly. However, a shift in research approach towards hippocampal brain slices in place of whole brain slices was required to improve model reliability and sensitivity. Formal weekly meetings between scientists in the fields of neurobiology (Dr. Simon's Group) and engineering (Dr. Bottlang's Group) ensure continuous exchange of results and coordination of future strategies. Frequent feedback from neurobiologist proofed crucial to adapt the initial injury model to the requirements of a tissue culture laboratory.

PI: Thomas D. Brown
Orthopaedic Biomechanics Laboratory
University of Iowa
2181 Westlawn
Iowa City IA 52242
319-335-7528
tom-brown@uiowa.edu

PROJECT TITLE: Nonlinear Computational Biomechanics of the Hip

PARTNERS' NAMES AND AFFILIATIONS: Michael G. Conzemius, DVM, Ph.D., Iowa State University; Robert A. Poggie, Ph.D., Implex Corporation; Richard A. Brand, M.D., John J. Callaghan, M.D., J. Lawrence Marsh, M.D., Stuart L. Weinstein, M.D., University of Iowa

GRANTING INSTITUTE: NIAMS

ABSTRACT
Disorders of the hip comprise a substantial fraction of current musculoskelatal disease burden. Complex nonlinear mechanical phenomena pervade many aspects of treatment of hip disease and injury, including total hip arthroplasty, intra-articular fractures, osteonecrosis, and developmental dysplasia. While bioengineering capabilities exist – in principle – to quantify key mechanical factors influencing treatment outcomes in these areas, contemporary clinical decision-making still rests almost entirely on subjective empirical experience. This Bioengineering Research Partnership (BRP) brings together the capabilities of an experienced computational biomechanics research group, four senior orthopaedic hip surgeons, a veterinary research orthopaedist, and an industry-based materials scientist, in order to advance the state of the art in biomechanically grounded management of disorders and injuries of the human hip. The central focus of the research Partnership lies in applying nonlinear finite element formulations to address as-yet-unquantified mechanical phenomena that are clinically recognized as being crucial to patient outcome. Building on previous and ongoing finite element work, new computational formulations will be developed to tackle nonlinearities currently limiting the accuracy of numerical simulations in five clinically important areas of hip surgery. The first two areas involve leading complications of total hip arthroplasty. First, as regards abrasive wear of polyethylene, we propose to incorporate local directionality of femoral head counterface motion in computing wear rates with a sliding-distance-coupled contact finite element formulation. Second, as regards dislocation, we propose to introduce soft tissue tethering into a large-displacement sliding contact model of resistance to dislocation. The third area involves intra-articular fractures of the acetabulum: estimating residual cartilage contact stress elevations accompanying attempts at surgical restoration of articular surface congruity. The fourth area involves osteonecrosis: computationally characterizing a new animal model (the emu) which unlike previous animal models progresses to human-like femoral head collapse, and using that model for *in-vivo* testing of computationally optimized placement of a novel head-preserving implant device. The fifth application area involves surgical management of developmental hip dysplasia: using novel mesh pre-processing techniques to quantify improvements of intra-articular contact stress achieved by pelvic osteotomies. This Partnership will bring together a critical mass of engineers and surgeons, to achieve clinically grounded advances in nonlinear numerical simulations of surgery of the hip.

STATUS OF THE PARTNERSHIP
On cross-over polyethylene wear (SA1), using a combination of experimental and computational methods, we have successfully mapped the direction-dependence of volumetric wear both for conventional and cross-linked polyethylene. We have identified several corresponding global surrogate metrics of wear rate. Also, we have compiled white-light interferometer mappings of scratch damage on surgically retrieved total hip femoral components, for which corresponding acetabular volumetric wear

data have been measured. It remains now just to run global finite element (Archard) wear simulations for these roughening-mapped femoral heads. On total hip dislocation (SA2), we now have a working 3-D FE model of dislocations incorporating full capsule representation (hyperelastic constitutive formulation), including wrap-around effects. The data from this model show a dramatic increase in dislocation resistance (nearly three-fold higher) as compared to an otherwise similar hardware-only construct. While it is gratifying that this technically-difficult model augmentation turned out to have such a pronounced effect, this in effect re-writes the landscape in dislocation FE analysis, and indicates that the entire existing body of hardware-only dislocation literature needs to be re-examined. Our voxel-based contact finite element formulation is fully operational. Besides our ongoing BRP work with acetabular fractures (SA3) and with developmental hip dysplasia (SA5), we have ported this formulation to the ankle. An expedited contact formulation using discrete element analysis, potentially suitable for intra-operative applications, has also been piloted for transtectal acetabular fractures. Our work with the emu model (SA4) has included developing a cryo-insult probe to create segmental lesions, a thermal finite element model to assess the distribution of osteolytic critical isotherms, and an image analysis routine automatically histologically quantify empty (dead) versus occupied (live) osteocyte lacuna, thus mapping the zone of osteonecrosis. Building blocks created for our emu structural finite element model have included cortical and cancellous bone material property measurements, joint contact force measurement, contact stress distribution measurement, and CT voxel-based meshing. The full three-dimensional FE model shows pronounced variation of lesion collapse propensity, depending on site and size of the lesion. Our relationship with the College of Veterinary Medicine at Iowa State University remains strong, and has now deepened to include a more definitive 3-dimensional analysis of emu hip joint loading during gait.

ISSUES

Still none. This BRP grant has absolutely fulfilled our goal of facilitating incubation of new computational formulations for articular joint biomechanics, which in turn have provided a springboard for innovative applications involving unsolved clinical problems in orthopedic surgery of the hip, and now other joint as well. Now just past the beginning of our 5th and final project year, we anticipate that there will be some carry-over funds, and that we probably will apply for a no-cost extension. However, we do not plan to re-compete for additional BRP funding in this particular area. This is because a new partnership research opportunity has arisen in a different area (wear of total disk replacement implants), an emerging problem of very high societal impact, and that we believe merits more urgent prioritization within our overall laboratory research program.

PI: Thomas S. Buchanan, Ph.D.
University of Delaware
Center for Biomedical Engineering Research
Department of Mechanical Engineering
126 Spencer Laboratories
Newark, DE 19716 USA
T: 1-302-831-2423
F: 1-302-831-3466
buchanan@me.udel.edu
www.cber.udel.edu

PROJECT TITLE: FES and Biomechanics: Treating Movement Disorders

PARTNERS' NAMES AND AFFILIATIONS:
Department of Mechanical Engineering: Thomas S. Buchanan, Sunil Agrawal, Kurt Manal
Department of Physical Therapy: Stuart Binder-Macleod, John P. Scholz, Katherine S. Rudolph

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

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

STATUS OF RESEARCH AND PARTNERSHIP

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

Biomechanical modeling: We have developed a biomechanical model of the ankle to use for this project. This model characterizes the morphology and force generating capacity of the musculature spanning the ankle. We have developed techniques to obtain parameters for the model from MRI and ultrasound to make it subject-specific. The model also allows us to estimate muscle forces from EMG signals during dynamics tasks. Our next step is to use this model to predict muscle activation patterns during gait, which can then be input to an FES protocol.

FES: We are developing mathematical models to predict changes in muscle force in response to changes in stimulation intensity. Also, we are designing and constructing hardware needed to allow computer control of the both the timing and intensity parameters of the stimulation trains. Our hardware modification now allows us to control both the pulse duration and interpulse interval of each pulse within each stimulation train. This ability allows us to control both the rate and number of motor units activated within each train. We have also created a series of experiments using patients who have sustained strokes and age matched control subjects that will allow us to test the applicability of our models to the plantar- and dorsi-flexor muscles of stroke patients.

Robotics: We are developing gravity-balanced leg orthoses for the human leg that can fully or partially balance the leg over its range of motion. These orthoses will be used in clinical studies of subjects with stroke to modify their walking patterns. The underlying theory of gravity balancing of the leg applies to three degrees-of-freedom (DOF) motion of the leg at the hip, one DOF flexion and extension at the knee, and one DOF ankle dorsi and plantar flexion was developed. The first prototype was targeted at two DOF motion of the leg in the sagittal plane, i.e., single DOF flexion and extension at the hip and knee. It verifies the conceptual idea of gravity balancing. This design assumed that the spring force is zero when the two ends of the spring coincide. A parallel theory and a prototype was developed when the spring has a nonzero initial length. We also developed the theory and design of a gait corrective orthosis using cam-follower theory using normal motion of the hip and knee during a walking cycle

ISSUES

There are no administrative issues that have arisen in regards to our partnership. In regards to the science, we have decided to reconsider the use of a treadmill in this study of human gait. We believe that a more realistic gait may be obtained during ground-based walking and have devised a means to accomplish that goal using a robot that would move with the person. This is a minor change to the goals of the project, but represents a major change in the robot design. However, this is coming along very well.

PI: Zhongping Chen, Ph.D.
University of California, Irvine
Biomedical Engineering Department and Beckman Laser Institute
1002 Health Science Road East
Irvine, CA 92612
Phone: 949-824-1247
Fax: 949-824-8413
Email: zchen@bli.uci.edu
Website: chen.bli.uci.edu

PROJECT TITLE: Optical Biopsy Using MEMS Technology

PARTNERS' NAMES AND AFFILIATIONS: Zhongping Chen (Biomedical Eng.)
G.-P. Li (Elec. Eng. and Comp. Sci.)
Mark Bachman (Elec. Eng. and Comp. Sci.)
Kenneth Chang, M.D. (College of Medicine)
Norman Tien, Ph.D.(Elec. and Comp. Eng., UC-Davis)

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

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

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

STATUS OF RESEARCH AND PARTNERSHIP

In the second year of this project, significant progress has been made in the advancement of functional OCT technology, silicon and non-silicon MEMS probe devices.

Following the first year's success in the demonstration of high resolution sub-cellular OCT imaging using supercontinuum generation from photonic crystal fiber pumped by a Ti:Saphire laser, the second year focused on the development of a portable broadband light source so that high resolution system can be used in a clinical setting. We have successfully developed a compact fiber femtosecond laser. We are currently testing and optimizing the fiber laser to pump photonic crystal fiber and other nonlinear fiber for supercontinuum generation. The third year will focus on optimization of the beam profile and development of a portable high resolution OCT system for clinical testing.

Several advances have been made in MEMS endoscopic probe. We developed the first rotational MEMS scanning probe. This novel probe was designed and constructed using a 1.9 mm MEMS motor. The new MEMS OCT probe design eliminates the need to couple the rotational energy from the proximal to distal end of the endoscope. Because this MEMS scanner does not require the coupling of a rotational single mode fiber, high scanning speed is possible while eliminating unstable optical signals due to nonuniform coupling. We have tested the MEMS rotational probe in animal studies and demonstrated its capability for in vivo imaging. The third year will focus on optimizing the probe design, packaging, and system integration.

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

ISSUES

We are working with UCI IRB for the approval of the human subjects protocol. One of the concerns the committee raised is that they would like to see some animal testing results, which we have just accomplished this year. In our proposal, the human subjects will not be involved until the fourth year. Therefore, we have sufficient time to obtain IRB approval before the start of clinical testing in the fourth year.

The original focus of the partnership is the development of endoscopic OCT using MEMS based probes for cancer diagnosis in the GI tract. One issue we face is that the technology we developed also attracts a lot of clinicians from other specialties that would like to use the device. It is difficult to accommodate most of these requests with only one system developed from this grant.

PI: Cheung, Alfred K., M.D.
Dialysis Program, 85 North Medical Drive East
Department of Medicine
University of Utah
Salt Lake City, UT 84112
T: 801-581-6427
alfred.cheung@hsc.utah.edu

PROJECT TITLE: Prevention of hemodialysis vascular access stenosis

PARTNERS' NAMES AND AFFILIATIONS:
Dr. Ramesh Rathi (MacroMed, Inc,)
Dr. Steven Kern (Department of Pharmaceutics, University of Utah) Dr. Gregory Burns (Animal Research, University of Utah)
Dr. Dennis Parker (Department of Radiology, University of Utah)
Dr. David Weinstein (Scientific Computer Institute, University of Utah)
Dr. Brett Milash (Department of Informatics, University of Utah)
Dr. Carie Phillips (Department of Pathology, Indiana University)
Dr. Jon Klein (Core Proteomics Laboratory, University of Louisville)

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

ABSTRACT
Neointimal hyperplasia is a frequent cause of stenosis in blood vessels and is commonly observed in post-angioplasty coronary arteries and hemodialysis vascular accesses. In native arteriovenous (AV) fistulae and polytetrafluoroethylene (PTFE) grafts for hemodialysis, the stenosis is usually focal and occurs at the AV or graft-venous anastomosis. Effective strategies for the prevention of stenosis are lacking. We hypothesize that local delivery of anti-proliferative drugs and anti-growth factor antibodies using a novel drug delivery system, ReGel®, will inhibit neointimal hyperplasia associated with native AV fistulae and PTFE grafts. ReGel is an injectable, thermosensitive copolymer designed for local, sustained-delivery of drugs.
This is a multidisciplinary approach to address the following specific aims: (1) To examine the efficacy of two anti-proliferative drugs (dipyridamole or paclitaxel) and anti-platelet derived growth factor (PDGF) antibodies alone or in combinations in the inhibition of growth of human or canine vascular smooth muscle cells (SMC). These in vitro studies will set the stage for animal studies in this proposal and potential clinical trials in the future. (2) To study the release kinetics of the anti-proliferative drugs and anti-PDGF antibodies from ReGel in vitro and their transport kinetics across explanted native AV fistulae and PTFE grafts. The transport characteristics of the drugs and antibodies in ReGel applied to the perivascular area of the native AV fistula and PTFE graft around the venous anastomosis will then be evaluated in whole dog experiments. Comparisons of mathematical model predictions with results from these experiments will help optimize the therapeutic dose of drugs and antibodies and conditions for delivery by ReGel in vivo. (3) To examine the efficacy of the anti-proliferative drugs and anti-PDGF antibodies delivered by ReGel in inhibiting neointimal hyperplasia in dog models of native AV fistula and PTFE graft.
Successful development of this technique will provide a novel approach of local drug delivery to prevent neointimal hyperplasia and stenosis in blood vessels. Furthermore, the results will provide the basis for local delivery of drugs and proteins of interest to a variety of tissues.
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STATUS OF RESEARCH AND PARTNERSHIP

Specific Aim 1: The two drugs that we have been concentrating on in vitro during the last year were dipyridamole and imatinib. Imatinib is an inhibitor of the phosphorylation of tyrosine kinase, which initiates the intracellular signaling pathway for PDGF receptor (PDGFR). Because of its simplicity and relative low toxicity, imatinib appears to be an ideal inhibitor of PDGF effect for in vivo applications. In addition to vascular SMC, adventitial fibroblasts appear to be an important participant of the intimal hyperplastic process. Therefore, we have been examining the effects of anti-proliferative drugs on both vascular SMC and fibroblasts in vitro. The results so far show that human dermal fibroblasts behave differently from human aortic SMC in their intracellular signaling response to PDGF and inhibition by imatinib. Other drugs, such as inhibitors of NADPH oxidase, are also being explored for potential in vivo applications.

Specific Aim 2: We have established in vitro models for the examination of drug transport kinetics across unused PTFE grafts, grafts explanted from experimental pigs, native porcine arteries and native veins. Preliminary results show that the permeabilities and partition coefficients for dipyrdamole vary greatly (up to 20-fold and 7-fold difference respectively) among the different types of vessels, with native arteries having the lowest permeability and explanted grafts having the highest partition coefficient. A subproject of this aim deals with the design of specific drug delivery systems. In order to enhance the delayed and sustained-release properties of the systems, we have been experimenting with the combination of ReGel with poly(lactide-co-glycolide) (PLGA) microspheres. The incorporation of dipyrdimale into the microspheres changed the release kinetics of dipyridamole from ReGel significantly, the tempo of which was dependent on the molecular weight of the PLGA. PLGA polymers with the highest molecular weights delayed the initial burst and prolonged the release of dipyridamole substantially.

Specific Aim 3: A major change in the last one year was the switching of the canine model to the porcine model of PTFE graft stenosis. We have completed a series of experiments in the canine model and have demonstrated the efficacy of local sustained-release of paclitaxel delivered by ReGel in inhibiting the development of intimal hyperplasia at the graft stenoses. However, the development of hyperplasia was eratic and slow in the dog. In some instances, the hyperplasia in the grafts that did not receive drug treatment was only mild, even at eight weeks. The use of paclitaxel was also plagued by impaired healing of the wound overlying the treated anastomoses. Because of these two problems, we have decided to test other drugs in the porcine model. A bilateral carotid artery-to-jugular vein PTFE graft model has already been established in the pig in our laboratory. Moderate neointimal hyperplasia was found at 14 days and severe hyperplasia was found at 21-28 days after graft placement. Further, there was intense staining for C-reactive protein on the SMC in the hyperplastic lesions.

ISSUES

There are no major issues, except for the efforts and adjustments required for establishing the new animal model and new collaborations with the various new partners.

PI: Shu Chien, M.D., Ph.D.
Department of Bioengineering
University of California, San Diego
9500 Gilman Drive
La Jolla, CA 92093-0412.
T: 858-534-5195.
shuchien@ucsd.edu

PROJECT TITLE: Molecular Basis of Endothelial Remodeling by Flow

PARTNERS' NAMES AND AFFILIATIONS: Dr. Jun-Lin Guan, Cornell University (Ithaca, NY). Dr. Martin Schwartz, University of Virginia (Charlottsville, VA)

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

ABSTRACT: Hemodynamic forces regulate the structure and function of the blood vessel wall. Vascular endothelial cells (ECs) are exposed to shear stress, the tangential component of the hemodynamic forces acting on the vessel wall. ECs in the straight part of the arterial tree are subjected to laminar flow with high shear stress, whereas cells in the bends and bifurcations are under disturbed flow patterns with low shear stress. Our **hypothesis** is that the preferential localization of atherosclerosis in the branch points of the arterial tree and the sparing of the straight parts can be related to the different molecular responses to these flow patterns. The laminar flow in the straight part of the vessels is anti-atherogenic by arresting the EC cell cycle. In contrast, disturbed flow at branch points is pro-atherogenic by increasing EC proliferation. Laminar flow also enhances the repair of the dysfuntional endothelium by augmenting EC migration, whereas disturbed flow retards the repair by inhibiting cell migration. We will test our hypothesis that laminar flow and disturbed flow activate different molecular signaling pathways to result in the expression of unique sets of genes, thus leading to the opposite functional consequences of anti-atherosclerosis and pro-atherosclerosis, respectively. The research design has three Specific Aims. In **Specific Aim 1**, we will establish the molecular basis of the arrest of EC cell cycle by laminar flow and the enhancement of the EC proliferation by disturbed flow. In **Specific Aim 2**, we will elucidate the molecular mechanisms by which EC migration is modulated by laminar and disturbed flows. In **Specific Aim 3**, we will identify the genes regulated by laminar flow and disturbed flow by using DNA microarray chip technology, with the aim of guiding in-depth studies on the flow-responsive genes that modulate EC growth arrest, proliferation, and migration. The proposed research involves partnership among scientists with expertise in vascular biology, physiology, biomechanics, bioengineering, bioinformatics, cell biology, and molecular biology. This interdisciplinary research program will allow us to elucidate the molecular basis of flow-induced modulation of EC turnover and migration, which are two important functions for vascular remodeling. The results will serve to generate new knowledge on mechano-transduction and vascular biology, provide new understanding of the molecular and biomechanical bases of pathogenesis of vascular disorders such as atherosclerosis, and help to develop new therapeutic strategies.

STATUS OF RESEARCH AND PARTNERSHIP:
We have made excellent progress in research under the three Specific Aims with valuable collaborations with our partners.

Studies on interplays between integrin and Flk-1 show that integrin is upstream to Flk-1 in their shear activation and that they share common downstream signaling. Integrins mediate the shear-activation of the serum responsive element binding protein SREBP-1 through the Rho-p160ROCK pathway. Collaborative study between University of Virginia/Scripps Research Institute and UCSD under this BRP has shown that equibiaxial stretch causes a uniform inhibition of membrane ruffle formation through

deactivation of Rac and that uniaxial stretch inhibits ruffling only along the sides while stimulating ruffling at the front and rear. Fluorescence activation indicator for Rho proteins (FLAIR) studies show a redistribution of the activated Rac to the leading edge parallel to tension, suggesting that the regulation of Rac activity by tension may be important for cell motility, polarization and directionality of movement. FLAIR on ECs subjected to shear stress shows that Cdc42 and Rac are polarized in the flow direction. The localized activation of Cdc42 is required for the shear stress-induced MTOC reorientation. The localized Rac activation is mediated by the shear-induced new integrin-ECM binding. The spatially organized activation of the small GTPases provides spatial information for shear-induced cell alignment.

Shear stress increases lamellipodial protrusion and EC migration. Paclitaxel (taxol) reduces the speed of migration, inhibits lamellipodial protrusion, blocks the flow-induced elongation and alignment, and decreases the shear-induced Rac activation. Under static and shear conditions, dominant negative mutant of Rac1 inhibits lamellipodial protrusion and cell migration. Active mutant of Rac1 induces lamellipodia in all directions and attenuates the shear stress-induced migration, suggesting that polarized lamellipodial protrusion and an appropriate level of Rac activity are important for cell migration.

Laminar flow promotes EC wound healing compared with the flow reattachment region under disturbed flow, which enhances the formation of focal adhesion complex. The tyrosine kinase inhibitor genistein inhibits wound healing under disturbed flow through the inhibition of junction dissociation. The Rho inhibitor C3 inhibits wound healing in both laminar and disturbed flows through the disruption of focal adhesions..

Microarray study of gene expression of human aortic ECs shows that 24 hr laminar shearing (12 dyn/cm^2) down-regulates several genes related to inflammation and EC proliferation, indicating that long-term shear keeps ECs in a non-inflammatory and non-proliferative state. Several genes involved in EC survival, angiogenesis, and vascular remodeling are up-regulated.

Collaboration with Dr. Guan at Cornell University have shown that FAK complex formation with Src, PI3K and other molecules in focal adhesions causes differential regulation of cell migration and cell cycle. DNA microarray studies on cells expressing wild-type FAK, dominant-negative FAK mutant □C14, or control Mock cells lead to the identification of a number of FAK up-regulated proteins (FURPs), including the Krüppel-like transcription factor KLF8. KLF8 protein expression is up-regulated by FAK expression, and down-regulated by □C14 expression. KLF8 expression is increased by cell adhesion to fibronectin and requires both FAK/Src and FAK/PI3K complex formation. These results indicate that KLF8 gene is a specific target of FAK. Induction of KLF8 in NIH3T3 cell lines results in a doubling of BrdU incorporation, indicating that KLF8 is a positive regulator of cell cycle progression through the G1 phase. Inhibition of endogenous KLF8 expression by siRNA reduces the FAK-induced stimulation of cell cycle progression.

In the rabbit aortic tree, *en face* mapping of EC nuclei orientation, which reflects the direction of flow streams, showed two main directions: longitudinal and spirohelical. The juxtaposition of these two types of flow streams results in alternations of high shear and low shear regions (i.e., the creation of high spatial shear gradients). These areas, as well as the branch regions, are associated with high rates of mitosis, cell death, albumin leakage, and expression of monocyte chemotactic factor 1 (MCP1). These results indicate the importance of complex flow patterns in modulating cell signaling and functions.

ISSUES: The P.I. did not receive e-mail notifications from the NIH about the BRP events a year ago because of the use of a wrong e-mail address. This has been corrected and now notifications are received regularly.

PI: E. Antonio Chiocca, M.D. Ph.D.
Department of Neurological Surgery
The Ohio State University Medical Center
N-1017 Doan Hall
410 W. 10th Avenue
Columbus, OH 43210
T: 614-293-5444
F: 614-293-4281
Email: Chiocca-1@medctr.osu.edu

PROJECT TITLE: Interdisciplinary Tumor Complexity Modeling.

PARTNERS' NAMES AND AFFILIATIONS: Thomas S. Deisboeck (**Co-PI**; Massachusetts General Hospital), David A. Weitz (Harvard University), Michael E. Berens (Translational Genomics Research Institute), Leonard M. Sander (University of Michigan).

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

ABSTRACT
The prognosis of patients suffering from malignant brain tumors remains dismal, largely due to the ability of glioma cells to extensively invade brain parenchyma. We hypothesize that such tumors behave as *complex dynamic, adaptive and self-organizing biosystems, whose behavior can be modeled and predicted*. Our specific aims are thus as follows: **In Aim 1**, we are determining and correlating validated changes in gene expression profiles with changes in extracellular environments. **In aim 2**, these correlative measures will be used to derive models predictive of brain tumor development and invasion, leading, ultimately, tot heir validation and confirmation with actual patient-derived imaging data.

STATUS OF RESEARCH AND PARTNERSHIP
(1). Infrastructure Development: We have set up a website with both general access (http://brptumor.org) and username/password protected areas http://brptumor.org/WG/. The former was to allow posting and sharing information about the project to the general public. The latter site has secure FTP (safeTP) and SSH/SFTP access for project members that mirrors the Public/Private web files where members can put up information about on-going projects, links to Project Member's email, and web information. We also established a ListServ Communications System (BRPtumor@brain.mgh.harvard.edu) with web enabled archive posting system for email based communications between project members. **(2). Scientific Progress against aims. Aim 1:** We have standardized the growth of several human glioma cells lines (U87, U87dEGFR, and U251) in the three-dimensional spheroid system. We have observed reproducible patterns of growth and infiltration, dependent on the cell line used, and we have compared these to the growth observed with neurospheres derived from mouse ES or neural progenitor cells. We have found that addition of EGF and FGF to the gels promote proliferation but inhibit invasion of glioma and neural stem cells into surrounding matrigel matrix. We have also started to determine the effects of the surrounding matrix on tumor growth and invasion. The subcontractual collaboration with **David A. Weitz, Ph.D.** (Division of Engineering and Applied Science and Department of Physics, Harvard University), is studying the effect of a collagen I matrix in which the tumor spheroid can grow and through which cell invasion can take place in three dimensions. We monitor cell-matrix interactions using a novel approach that couples confocal reflectance imaging of collagen fibers and Coherent Anti-stokes Raman Spectroscopy (CARS) imaging of cells. Using this approach we have been able to study the local cell-matrix mechanical interactions mediating invasion. We find that invasive cells deform and remodel the surrounding matrix, exerting significant forces on the order of 10pN. In addition to the capability for high-resolution imaging of these interactions, a primary advantage of this model system is that it allows us to change the tumor's mechanical

environment and study the response. In particular, the collagen matrix mesh size can be changed by a factor of 5 and the elastic modulus by more than an order of magnitude by changing the collagen concentration. We are currently studying the growth dynamics of tumor spheroids in such matrices to elucidate the role played by local structural and mechanical parameters of the tissue environment. Because we plan to further determine how the genetics of the tumor models are impacted by the surrounding matrix environment, the subcontractual agreement with **Michael E. Berens, Ph.D.** (Neurogenomics Division, Translational Genomics Research Institute), is delineating the genomic and Functional Analysis of the multicellular tumor 3D Invasion System. Laser capture microdissection (LCM) of invasive and non-invasive U87WT and U87ΔEGFR cell spheroids was performed. Subsequently RNA was isolated, amplified, labeled and hybridized against universal reference RNA onto human oligonucleotide microarrays. Preliminary data analysis of U87WT demonstrates that the samples taken from the core display significant correlation in their global gene expression profiles amongst themselves and the samples from the invading edge display significant correlations in their global gene expression analyses amongst themselves. Further analysis on this genomic dataset will be performed by multivariate statistics and pattern recognition techniques aiming to identify genes differentially regulated between invasive and stationary cells within each cell line as well as in between the two cell lines exhibiting two distinct invasive phenotypes. **Aim 2:** Progress against this aim has also been obtained. **Thomas S. Deisboeck, M.D.** (Complex Biosystems Modeling Laboratory, Harvard-MIT Martinos Center for Biomedical Imaging, Massachusetts General Hospital) is developing novel computational models capable of simulating the mechanistic spatiotemporal dynamics of malignant brain tumors, and *top-down* integrating three different scales, i.e., macroscopic, microscopic, and molecular dynamics, under one unified framework. A 2-D *complex-systems* model has been created using a discrete "*agent*-based" approach with the individual cancer cell as the smallest unit of observation, which will perform a local *biased* search and invade a matrix based on pathways of "least resistance and highest attraction". We found that these virtual tumo cells can achieve maximum velocity at *less* than 100-percent search precision, indicating a facilitating role for *noise*, and that there is increase in tumor cell velocity towards the nutrient-abundant region of a surrounding gel. We also found a *positive* correlation between the fractal dimensions of the tumor surface and its expansion velocity and the *time-series* profile averaged across all cellular phenotypes was found to contain *more* predictive power than separate gene-expression profiling for each distinct phenotype. Encouraged by these results, we are currently developing a more complex *gene-protein network* for the phenotypic 'switch' between proliferation and migration mediated by the Epidermal Growth Factor Receptor (EGFR)-dependent *cell-signaling network*. Ultimately, this intracellular module should also be incorporated in the subcontractual studies by **Leonard M. Sander, Ph.D.** (Randall Laboratory of Physics, University of Michigan), who has been modeling tumor cell invasion and growth using continuum methods. This should provide an overall picture of the development of the *in vitro* tumors that we observed in earlier work. The general picture is that we have two `species' of tumor cells: proliferative, which we call *s*, and invasive, *a.* Coupled reaction-diffusion equations were written for the development of tumor (*s*), conversion of *a* to *s*, and chemotactically controlled streaming of the (*a*) cells away from the tumor. Invasion is driven by the gradient of some small molecule such as glucose or a growth factor, such as TGF-α which is consumed by the central tumor. Chemotaxis fits the previously observed growth of the invasive zone.

ISSUES

So far, we have not encountered and programmatic or scientific issues that have impeded our progress.

PI: Churchill, Bernard, M.D.
David Geffen School of Medicine at UCLA
Department of Urology
Box 951738
Los Angeles, CA 90025-1738
T: 310-206-9718
F: 310-206-9726
bchurchill@mednet.ucla.edu
www.urosensor.com

PROJECT TITLE: Uropathogen Detection Using DNA Biosensors

PARTNERS' NAMES AND AFFILIATIONS:
David Bruckner (Pathology & Laboratory Medicine), Vincent Gau (GeneFluidics, Inc.), Warren Grundfest (Biomedical Engineering), David Haake (Infectious Disease), Chih-Ming Ho (Mechanical and Aerospace Engineering), Elliot Landaw (Biomathematics), Joseph Liao (Urology), Edward McCabe (Pediatrics)

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

ABSTRACT
Urinary tract infection 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 a system for the genotypic detection and species-specific identification of uropathogens within a time frame (30 minutes from sample collection to readout) that would enable point-of-care diagnosis and treatment. The focus of this proposal is to develop a self-contained microbial pathogen detection device and to examine its performance using clinical urine samples. Research at UCLA has provided two key technological advances that make development of an uropathogen sensor feasible. The first is microfluidics for sample processing. The second is an electrochemical microsensor that allows ultrasensitive detection of specific DNA-RNA or DNA-DNA hybridization events, with the need for target amplification step such as PCR. Specific Aim 1 describes how microfluidics studies will be applied to development of a filter for uropathogen concentration, micromixing for processing of uropathogen nucleic acids, and washing of the sensor surface. Specific Aim 2 involves fabrication of the microsensor array, development of a funtionalized self-assembled monolayer, and testing of oligonucleotide probes for electrochemical detection of uropathogen rRNA and mRNA on the microsensor surface. Specific Aim 3 will involve integration of the microfluidics and sensor components and testing of its analytic validity on simulated and actual urine specimens. Specific Aim 4 will involve fabrication of sufficient number of device to test the association between urosensor results and clinical correlates of urinary tract infection.

STATUS OF RESEARCH AND PARTNERSHIP:
Project Organization. We are continuing to utilize the organization structure that was put into place during the first year of the project, including monthly research meetings, monthly administrative meetings, and posting of electronic reports on our website.
Microfluidics. We have successfully developed a UCLA cross-flow filter to separate and concentrate a minute amount of bacterial cells from a large amount of fluid. The cross-flow filter is based on polycarbonate (PCTE) membrane housed in acrylic plastic. Particles larger than the 0.45 μm pore size (e.g. bacteria) do not pass through the membrane. Our data show that the UCLA cross-flow filter is effective in the concentration of E. coli. The performance of the cross-flow filter can satisfy the design requirement for the sample preparation system. We have also continued our efforts from last year to use

of dielectrophoretic (DEP) force to manipulate small particles at the microfluidics level. DEP force may be incorporated as both as a method of bacterial concentration and reagent mixing. We have designed and fabricated a DEP force biofilter in the UCLA Nanolab. An integrated array of electrodes was designed to create a non-uniform electric field that induces a dipole moment on any uncharged or conductive particle (such as a bacterium) in the fluid. When the dipole moment is induced, the particle is drawn down by the electric field and captured near the edge of the electrodes. The performance of the biofilter was tested with polystyrene micro-sphere particles suspended in deionized water and *E. coli* suspended buffer. We are currently designing a micromixer for reagent mixing for the sample preparation module.

Probe Design and Testing. The temperature dependence and kinetics of uropathogen species-specific probe hybridization were examined using a set of oligonucleotide probes based on species-specific regions of the 16S gene of the *Escherichia coli*, *Proteus mirabilis*, *Klebsiella oxytoca*, and *Pseudomonas aeruginosa*. A universal bacterial oligonucleotide probe and oligonucleotide probes specific for gram-positive and gram-negative organisms were also included in these studies. The oligonucleotide probes were able to discriminate among 16S genes derived from eleven different species of uropathogenic bacteria applied to nylon membranes in a dot-blot format. The kinetics of hybridization were extremely rapid. Significant binding of oligonucleotide probes to target DNA took place within 10 min and species-specificity of the probes was retained at room temperature. We have also designed and tested a set of species-specific DNA probes against *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterococcus* spp., and *Pseudomonas aeruginosa* on the electrochemical sensor array. A universal bacterial probe has also been designed and tested. The sensor array demonstrated rapid, species-specific hybridization in a time course consistent with the rapid kinetics of the dot blot hybridization studies. We have also demonstrated direct detection of uropathogens in unprocessed urine specimens using our species-specific probes and sensor.

Electrochemical Sensor Array. Considerable progress has been made by GeneFluidics in the sensor array fabrication process incorporating improved masking and gold deposition techniques and surface functional layer treatment in a semi-automated production line. This has resulted in production of a greater number of sensors with decreased variance and improved accuracy and consistency. Shelf life of the sensor up to six months has been confirmed by specific experiments.

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

PI: Mark G. Clemens, Ph. D.
University of North Carolina at Charlotte
Department of Biology
9201 University City Blvd
Charlotte, NC 28223-0001
T: 704-687-4040
F: 704-687-3128
mgclemen@email.uncc.edu
http://www.coe.uncc.edu/~rncoger/BRP/

PROJECT TITLE: ENGINEERING ASPECTS OF LIVER SUPPORT SYSTEMS

PARTNERS' NAMES AND AFFILIATIONS: Robin N. Coger, Ph. D. (Mechanical Engineering, UNC Charlotte), Charles CY Lee, Ph. D. (Mechanical Engineering, UNC Charlotte), Laura Schrum, Ph. D. (Biology, UNC Charlotte), Jian Zhang, Ph. D. (Biology, UNC Charlotte)

GRANTING NIH INSTITUTE/CENTER: National Institute of Diabetes and Digestive and Kidney Diseases (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
Overall, the partnership has continued to strengthen with significance progress being made on the aims of the grant. In addition, the partnership has allowed us to train several Ph.D. students in an interdisciplinary environment that relies on close collaborations between biomedical scientists and engineers.

The goal of Project 1 is to test the hypothesis that nutrient transport enhancement strategies improve overall cell function by decreasing spatial heterogeneity, thus improving nutrient to cell supply-demand ratios. In the current project year we have used our modified in vitro system to compare O2 transport through the collagen extraceullar matrix gel under acellular and cellular conditions. The results demonstrate that the O2 enhanced ECM is more effective than normal ECM in transporting O2 in both cases, thus suggesting that our enhancement method will aid in scaling up the cellular space of packed bed Bioartificial Liver designs. Work in the next project year extends this work to evaluating if our enhancement technique creates localized regions of either hyperoxia or hypoxia for hepatocytes packed within the Bioaritifical Liver. To evaluate if the

response of the hepatocytes to the increased O2 transport, work in the current project year included evaluating the oxidative stress metabolic levels of the hepatocytes in the enhanced and normal ECM systems. More specifically we are measuring reactive O2 radical and scavenger parameters (i.e., total glutathione content, catalase, glutathione peroxidase and glutathione reductase; as well as lipid peroxidase). The initial results suggest that total glutathione production in the enhanced system is typically improved over that in the normal system in time. This portion of the study will be continued in the next project year. Finally, now that O2 transport through packed bed systems seems to be significantly improved by our enhancement technique, it is necessary to evaluate if the technique has an effect on the permeability of the ECM to solutes in the media. To that end, we have recently begun experiments measuring the transport of fluorescent labeled dextran, of various sizes, through the normal and enhanced ECM.

The goal of Project 2 is to test the hypothesis that heterotypic interactions between hepatocytes and Kupffer cells maintain and enhance hepatocyte viability and function. During the course of this year, the surface characterization of the micropatterned surfaces have been completed; and adjustments have been made in our micropatterning method and the ingredients of our nutrient medium to meet the needs of both cell populations. We have now successfully engineered several micropatterned surface configurations using polydimethylsiloxane membrane templates and used them for the co-culture of hepatocytes and Kupffer cells. We are currently comparing hepatocyte function results for several ratios (e.g., 10:1, 5:1, and 2:1) of micropatterned hepatocytes to Kupffer cells. The results demonstrate that the presence of Kupffer cells in the hepatocyte system has a definite effect on hepatocyte viability and function. Whether this effect is due to physical contact or soluble factors is currently being evaluated by comparing the micropatterned culture results to experiments using transwell plates. In the next grant year we plan to also evaluate the effects of the micropatterned co-culture configurations on Kupffer cell function (e.g., phagocytosis).

The goal of Project 3 is to develop hypothermic machine perfusion (HMP) as a viable strategy for preservation of livers for transplantion. This project has advanced on multiple fronts. First, 24hr hypothermic machine perfusion (HMP) preservation experiments have shown that the endothelial cells are damaged as a result of the current method of HMP. Further experiments show that we can reduce this damage with oncotic support in the preservation solution. The results show that endothelial cell function is preserved better with HMP than simple cold storage at 24hrs. Second, we have shown that in the rat transplant model, non-heart-beating donor livers can be reclaimed and preserved for 5hrs. It appears that HMP is able to restore the ATP levels in the livers during preservation to near control levels. We suspect that this is an important component of HMP of NHBD livers. Lastly, we have begun collaboration with Organ Recovery Systems, a company in Charleston, SC, in testing of their Liver Transporter (LTR) in the porcine model. Preliminary experiments show promise in preserving these livers for 24hrs.

Our goals for the coming year on Project 3 are to begin transplant studies of the 24hr HMP experiments, begin investigating the mechanism by which HMP recovers NHBD livers, and continue further testing of the LTR with Organ Recovery Systems. This year has produced 3 journal articles, 3 abstracts, and 2 papers in preparation.

ISSUES

The partnership continues to function remarkably well largely because of biweekly meetings where all partners, students, and technicians working on the projects are present. It continues to be a challenge to maintain an effective interface between the engineering and biological aspects of the projects, but the frequent interdisciplinary meetings have substantially improved the understanding of the biology on the part of the engineers and the engineering on the part of the biologists. The input of our clinical collaborator has also been extremely valuable for the continued focus of the projects on ultimate clinical application. Work supported by the partnership has already resulted in additional grants (one RO1 and one SBIR) and pending applications by the partners to support projects based upon the findings of the BRP grant and the collaborative relationships established in the partnership. The success of the partnership has also served as the focus for the planned establishment of an Institute for Biomedical Engineering Systems on the UNC Charlotte campus, which we expect to be established in the coming academic year.

PI: Edward D. Crandall
Keck School of Medicine
University of Southern California IRD 606
Department of Medicine
2020 Zonal Avenue
Los Angeles, CA 90033
T: 323-226-7923
F: 323-226-2738
ecrandal@usc.edu

PROJECT TITLE: Absorption mechanisms of peptide/protein drugs via lung

PARTNERS' NAMES AND AFFILIATIONS:
Kwang-Jin Kim, Ph.D. - Departments of Medicine and Biomedical Engineering, USC Keck School of Medicine and School of Engineering
Vincent H.L. Lee, Ph.D. - Department of Pharmaceutical Sciences, USC School of Pharmacy
Wei-Chiang Shen, Ph.D. - Department of Pharmaceutical Sciences, USC School of Pharmacy

GRANTING 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. In this proposal, our long-term goals are to elucidate the mechanisms for absorption of various classes of peptide/protein drugs across the alveolar epithelium (that affords a vast surface area and relatively low protease activity). Although pulmonary delivery of protein / peptide drugs in animal studies has been shown to yield much better bioavailability compared to oral delivery, absorption mechanisms and pathways are mostly undefined to date. Many bioengineering-related issues are associated with pulmonary drug delivery, including formulation of specific drugs, modes of delivery and transport mechanisms. Of these, we have delineated various transport mechanisms that facilitate absorption of peptide/protein drugs across alveolar epithelium using primary cultured rat alveolar epithelial cell monolayers as an in vitro model. Model proteins/peptides studied range from oligopeptides to proteins of biological importance (e.g., insulin, enkephalin, calcitonin, granulocyte-colony stimulating factor (GCSF), human growth hormone, and transferrin). Our research plan is subdivided into four major projects: i) investigate transcellular transport mechanisms (e.g., fluid-phase, receptor-mediated and/or adsorptive transcytosis) for absorption of model drugs across the alveolar epithelial barrier, ii) delineate the effects of physicochemical characteristics of model drugs on alveolar epithelial absorption, iii) elucidate strategies for enhancement of alveolar epithelial absorption of protein/peptide drugs via paracellular and /or transcellular routes (e.g., transient alteration of barrier properties), and iv) study enhanced receptor-mediated transcytosis of macromolecule drugs (e.g., conjugation with transferrin in the presence of trans-Golgi disruptors). Our collaborative investigation of pulmonary protein/peptide drug absorption, utilizing many different experimental approaches spanning cell biology to bio(chemical)engineering, promises success in providing pertinent information on advancing practical approaches to pulmonary drug delivery.

STATUS OF RESEARCH AND PARTNERSHIP
Projects 1 and 4: Our previous studies performed on rat alveolar epithelial cell monolayers (RAECM) demonstrated putative expression of neonatal IgG receptor (FcRn) and net absorption of IgG via FcRn-mediated transcytotic process(es). Recently, we investigated if FcRn expressed in rat lungs can be utilized for pulmonary absorption of IgG in vivo. Male Sprague-Dawley rats were administered 20 □g biotin-labeled IgG alone or 20 □g biotin-labeled IgG plus 220 □g unlabeled rat Fc in 200 □L saline via

an intratracheal microsprayer. Serum was recovered at 18hr post-administration via heart puncture (for comparison studies of maximal concentrations (Cmax)), or at regular intervals via tail vein (for pharmacokinetic studies). Serum IgG levels were determined by ELISA using streptavidin-coated microplates with peroxidase-labeled secondary antibody against rat IgG. Results show that inclusion of excess Fc in the instillate yielded a significant decrease in Cmax of biotin-labeled IgG transport (32.5 (1.9) ng IgG/mL of serum for the IgG group vs 13.4 (2.7) ng IgG/mL of serum for the IgG plus excess Fc group). Pharmacokinetic studies, based on quantitation of transported IgG by a method utilizing estimation of area-under-the-curve (AUC), revealed a significant decrease in bioavailability of IgG (AUC of 548 ng hr/mL for the IgG group vs 240 ng hr/mL for the IgG plus excess Fc group). These early findings indicate that systemic absorption of IgG via the lung is dependent on Fc-mediated processes.
Project 2: We studied expression of efflux pumps (e.g., P-glycoprotein and MRP1) and lung resistance-related protein (LRP), which play an important role in limiting drug absorption through epithelial barriers, using RNA obtained from RAECM grown on permeable supports for five days in the presence (to maintain type II cell-like phenotype) or absence (for progression toward type I cell-like phenotype) of KGF. For RT-PCR analysis, one set of primers was used each for mdr1b and MRP1, whereas LRP expression was determined using two different sets of primers. GAPDH was used as the housekeeping gene. A distinct 550-base pair (bp) band for MRP1 and a 738-bp band for LRP were noted in RT-PCR analyses of RNA obtained from type I-like cells. A different set of primers confirmed the existence of LRP in type I-like cells as a 439-bp band. However, these cells did not express mdr1 gene. By contrast, type II-like cells exhibited a distinct 700-bp band for mdr1 and 738-bp band for MRP1. LRP showed a very weak signal in type II-like cells with one set of primers and no expression with the other set. These data indicate that rat alveolar epithelial type I cells appear to express MRP1 and LRP, whereas type II cells seem to express mdr1b and MRP1. The differential expression pattern/profile of various efflux systems in alveolar epithelium is likely to play important roles in systemic drug absorption via the deep lung.
Project 3: We have recently observed that when a peptide drug (e.g., calcitonin or insulin) is dosed with conditioned media (CMII, a spent culture medium obtained from apical compartment of primary cultured rat type II-like cells), peptide transport is enhanced over that with serum-containing media (SM) or serum free media (SF), or conditioned media (CMI) obtained from cultures of type I-like cells. We therefore sought to investigate the factor(s) present in CMII that enhance peptide transport. Soluble factor(s) (whose molecular weight(s) are greater than 30 KDa) in CMII appear(s) to enhance peptide transport across monolayers of type II-like cells. The enhancing factor(s) do(es) not affect paracellular transport or fluid phase endocytosis, since respective transport rates for mannitol and horseradish peroxidase are not affected by CMII. It appears that activity of the enhancing factor(s) is highly temperature-sensitive, as it decreases significantly when CMII is pre-treated at 550C for 15 minutes. Based on these preliminary results, we hypothesize that protein(s) (e.g., surfactant protein A (SP-A)) produced by type II-like cells may be the enhancing factor(s) for peptide transport. Further studies are required to characterize such enhancing factor(s) for alveolar absorption of peptide drugs.

ISSUES

(1) PI and Co-Investigators participate in monthly meetings to develop overall strategy, stimulate further collaborations, and plan optimal directions for the four projects. Quarterly scientific meetings of all personnel involved in the projects are held, where each group presents its latest research findings. One of the Co-Investigators, Dr. V.H.L. Lee, has now left USC. Drs. Kim and Shen each carried out parts of the projects left behind by Dr. Lee.
(2) A monthly seminar series sponsored by the project leaders is held under the auspices of the USC Center for Drug Design/Delivery. Recent speakers include: J.A. Nolta (Washington U., 5/7/04), J.F. Pittet (UCSF, 6/4/04) and A.J. Hickey (U. N. Carolina, 6/25/04).
(3) We are planning to study mechanisms of absorption of peptide/protein drugs across the alveolar epithelial barrier. Effects of ethanol and other enhancing factors will be investigated for reversible enhancement of drug absorption. In vivo studies for absorption via lungs of intact animals are ongoing.

PI: Anders M. Dale, PH.D.
Dept. of Radiology
MGH-NMR Center
149 13th St., Rm. 2301
Charlestown, MA 02129
T: 617-724-9771
F: 617-726-7422
dale@nmr.mgh.harvard.edu

PROJECT TITLE: Spatiotemporal Brain Imaging: Microscopic & Systems Level

PARTNERS' NAMES AND AFFILIATIONS:
Dr. Lawrence Wald (MGH/Harvard/MIT), Dr. David Boas (MGH/Harvard/MIT),
Dr. Roger Tootell (MGH/Harvard), Dr. Anna Devor (MGH/Harvard),
Dr. Amiram Grinvald (Weizmann Institute, Rehovot, Israel),
Franz Schmitt (Siemens Medical Systems, Erlangen, Germany)

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

ABSTRACT
The last decade has brought revolutionary new techniques allowing imaging of the working brain in humans at the systems level. However, a large gap remains between the spatiotemporal resolution of tomographic techniques (fMRI, PET), and the circuit level where animal studies permit mechanistic neural models. It is the overall goal of this proposal to develop an integrated suite of technologies to bridge this critical gap. Two interrelated themes are found throughout this proposal: (1) to improve the spatial and temporal resolution of non-invasive technologies, which will enable direct imaging of discrete (e.g., column and laminar level) neural units which bridge the systems and cellular levels; and, (2) to clarify the mechanisms which relate the biophysics of neuronal activity to "observables" in our imaging measurements. The two key technologies to be developed are: (1) e*xtremely high resolution MRI and fMRI*, using very high strength gradients, phased-array coils, and other advances at 3T and 7T in non-human primates, and 9.4T in rats; and (2) *tomographic optical imaging*, increasing the resolution and physiological range using three different optical technologies: direct reflectance imaging, optical scanning microscopy, and diffuse optical tomography. These technologies will be validated against invasive "gold standard" techniques in studies of rat whisker barrel cortex and macaque visual cortex, and further applied to animal models of spreading depression in migraine and stroke. Each of these experiments is designed to allow us to serially step from more to less invasive, and move from systems where much is already known through to studies in humans, which have not heretofore been explored within the spatiotemporal domains our newly developed tools will afford.

STATUS OF RESEARCH AND PARTNERSHIP

Aim 1: Improve fMRI Spatial Resolution: The proposed MR technology development is intended to increase the spatial resolution of functional MR imaging as needed to allow the study of cortical columns and lamina in the rat somatosensory cortex and primate visual cortex. The primary approach was to develop pulse sequence methods, RF array coil technology and gradient coils for ultra-high field imaging (9.4T for rodents and 7T for primates).

The past two years has shown significant progress toward our goals of columnar and laminar imaging in primates in our horizontal bore (90cm dia.) 7 Tesla system. We have implemented and tested a 32 channel RF front end for the system and have tested 2, 4 and 8 channel receive-only primate arrays.

Testing of these coils on the anesthetized macaque has produced clear evidence of the ability of high field MRI to resolve cortical lamina.

We have also developed the technology needed for functional imaging of awake behaving monkey fMRI at 7T. This has included the training and reward system for use in the 7T scanner as well as eyetracking, which is used as a basis for the reward and a 1.25mm resolution isotropic EPI sequence. We are currently doing 3 studies a week in the 7T system on 2 trained monkeys for the retinotopic fMRI point-spread function aim.

For the rat imaging technology aim we have developed and tested the MR compatible piezo-electric whisker barrel cortex stimulator and rat stereotax for use in the 7T and 9.4T systems as well as single receive-only surface coils and 0.5mm isotropic resolution EPI sequences for the rat brain fMRI.

Aim 2: Improve the spatiotemporal resolution of optical imaging: Multi-spectral imaging of hemoglobin concentrations and oxygenation has been achieved by the construction of a filter wheel based illumination system, which is synchronized with the camera detection system. We imaged high spatial and temporal resolution 2D maps of changes in hemoglobin concentration and oxygenation due to functional changes such as whisker deflection and cortical spreading depression. Additionally we have developed laser speckle contrast imaging for high resolution imaging of cerebral blood flow.
In addition to the surface reflectance imaging methods, we have successfully developed and tested a laminar optical tomography (LOT) scanning microscope for depth-resolved imaging of hemodynamics.

Aim 3: Apply the new technology to image functional activation: Over the past two years we have carried out experiments to address the question of coupling between the hemodynamic signal and activity of neurons in rat somatosensory (Barrel) cortex using simultaneous electrophysiological and optical measurements. Using simultaneous multiwavelength optical and electrophysiological recordings, we have estimated a strongly non-linear neurovascular transfer function. We showed that with an increase in stimulus amplitude the hemodynamic response continued to increase beyond the saturation of cortical electrical activity. We also showed that cortical neuronal activity in a principal barrel was closely coupled to thalamic input from a corresponding barreloid. Therefore, the hemodynamic behavior cannot be explained by either local pre- or post-synaptic neuronal activity. Using simultaneous spectroscopic and speckle optical imaging we calculated the cerebral metabolic rate of oxygen consumption (CMRO2). In contrary to previous theoretical predictions, CMRO2 maps did not show better spatial localization than hemoglobin oxygenation maps.

Using improved coil technology and newly developed multi-echo sequences we have commenced a series of fMRI experiments in macaque V1 using event-related checkerboard stimulus of different intensities. The necessary SNR is achieved by our recent progress in motion-correction algorithms.

In collaboration with Amiram Grinvald, we have performed a number of preliminary voltage-sensitive dye experiments in behaving monkeys using the same experimental design as in fMRI studies. The analysis of these data is underway to determine the relationship between voltage-sensitive dye signals that are believed to reflect mostly sub-threshold neuronal activity, and hemodynamics measured by fMRI and intrinsic signal optical imaging.

ISSUES
None

PI: Peter F Davies
University of Pennsylvania
1010 Vagelos Research Laboratories
3340 Smith Walk
Philadelphia, PA 19104-6383
T: (215) 573 6813
pfd@pobox.upenn.edu

PROJECT TITLE: Cell and Molecular Studies in Cardiovascular Engineering

PARTNERS' NAMES AND AFFILIATIONS: Childrens Hospital of Philadelphia (CHOP)

GRANTING NIH INSTITUTE/CENTER: NHLBI

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

In year 3 (7/03-6/04), 34 peer-reviewed papers of work supported by the BRP were published or accepted for publication and a substantial number of abstracts were presented at major meetings.

STATUS OF RESEARCH AND PARTNERSHIP:

The BRP has an integrative partnership with Childrens Hospital of Philadelphia (CHOP) on the same campus.
A **Minority Supplement** was awarded to graduate student Amanda Lawrence 2003-6

Progress Year 3: **Substantial progress in all aspects of the program resulted in publications in leading and specialist journals. They include mechanotransduction investigations:**

(i) at the cellular and subcellular levels **both in vitro and in situ related to cytoskeleton, ion channels, membrane biophysics, integrin signaling/ECM, morphogenesis, (*J. Biol. Chem. x 2, Biophys. J. x 4, Am. J. Physiol. x 2, Circ. Res., Ann. Biomed. Eng. x 4, J. Physiol., Arter. Thromb. Vasc. Biol., J. Gen. Physiol., Cell Motility Cytoskel., J. Cell Sci., Langmuir, and Biochim. Biophys. Res. Comm*.).**

(ii) at the in vivo and ex-vivo level **of tissue investigations of blood vessels, heart valves, and site-specific therapy with publications in *J. Biomech. x 2, Gene Therapy, Biorheology, Ann. Biomed. Eng.,J. Controlled Release, J.Heart Valve Disease,* and *Am. J. Pathology*.**

Among several important highlights (lead investigator/s in parenthesis):

- **The subcellular live cell mapping of cytoskeletal strain in endothelial cells (Davies)**
- The of lateral cell-cell border location and extracellular/transmembrane domains in PECAM/CD31 mechanosensation (Davies)

- The induction of endothelial plasma membrane caveolae by shear stress (Davies)
- A new technique for the isolation of high quality RNA fron small regions of the endothelium in situ and its application to heart valves (Davies)
- Membrane cholesterol-actin interactions in mechanotransduction (Levitan)
- The molecular identification of Kir ion channels in endothelium (Levitan/Davies)
- The role of phosphatases in mechanotransduction (Weaver)
- Cell responses to substrate stiffness (Weaver/Janmey)
- Spatial-mechanical regulation of morphogenesis (Weaver/Hammer)
- Single molecule unfolding force measurements (Discher)
- Force measurements on membrane proteins in situ (Discher)
- Cell peeling dynamics (Discher)
- Force traction studies of endothelium (Hammer)
- Review papers in several areas of mechanotransduction (Davies, Janmey, Discher)
- The identification of molecular cues regulating ex vivo vein remodeling (Gooch)
- Matrix metalloproteinases in the pathology of heart valves (Levy)
- Valve stabilization and protection against calcification (Levy)
- Viral vector gene delivery for vascular disease (Levy/Diamond)
- Development of new photolytic materials for microcoil deployment and local release of materials (Diamond)

ISSUES

No significant negative issues. There are no barriers to group interactions of the participants; the institute helps facilitate this. Al groups are located close to each other. There are regular BRP investigators meeting to present and discuss data as well as integration of BRP work into the institute and departmental seminar series and chalk talks.

PI: Pedro J. del Nido MD
Department of Cardiac Surgery
Children's Hospital
300 Longwood Ave.
Boston, MA 02115
T: 617-355-8290
Pedro.delnido@tch.harvard.edu

PROJECT TITLE: Image Guided Intracardiac Beating Heart Surgery

PARTNER'S NAMES AND AFFILIATIONS:
Robert Howe PhD, Engineering and Applied Sciences, Harvard
Pierre Dupont PhD, Robin Cleveland PhD-Mechanical Engineering, Boston Univ.
Ivan Salgo MD, Philips Medical Systems, Andover, MA
Gerald Marx MD, John Triedman MD, Dept. Cardiology, Children's Hosp., Boston, MA,

GRANTING INSTITUTE: 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
In the first year 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 will aid navigation and instrument visualization. We have also begun the second phase of this project, which is to modify instruments and instrument surfaces to make them more ultrasound "friendly" and to minimize artifact. We have also started the development process for placing electromagnetic (EM) trackers on instruments and generating a coordinate system for instrument position with respect to the target anatomy. In the second year we will continue work in the latter area and begin to integrate the EM coordinate system with the ultrasound image coordinate system to permit overlaying the EM tracker generated field with US volume rendered data. As proposed, the initial developments will be evaluated with our in vitro tank set-up prior to animal experiments for in vivo validation. The most important engineering development by Philips which will occur in the next year, will be the development of a streaming board to export post-scan-converted data for external off-line analysis and other possible manipulations including calibration with the tracking equipment. Philips engineers will develop hardware streaming output of Live 3D voxel data, and hardware input to the SONOS 7500 to accept rotation matrix and image freeze. This development will enable offline, real-time image processing integration with electromagnetic tracking instrument position data

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 but some of the engineering developments planned for year one will need to be done in year two.

PI: Carlo J. De Luca
Boston University, NeuroMuscular Research Center
19 Deerfield Street, 4th Floor, Boston, MA 02215
Tel:617.353.9756
Fax:617.353.5737
cjd@bu.edu
http://nmrc.bu.edu, http://nmrc.bu.edu/technology/

PROJECT TITLE: Harnessing Motoneuron Activity: From Lab to Clinic

PARTNERS: Dr. Hamid Nawab (Boston University), Dr. Alex Adam (Boston University), Dr. Rick Roark (New York Medical Center), Dr. Mario Manto (Free University of Brussels)

GRANTING INSTITUTE: National Center for Medical Rehabilitation Research (NCMRR)

ABSTRACT
We propose to develop an automatic system for decomposing the electromyographic (EMG) signal into the constituent action potentials corresponding to the firing of individual motor units activated by motoneurons. The system will be an outgrowth of our existing rudimentary system, which over the past 20 years has enabled us to perform various novel investigations that have provided a variety of new insights into motor control. However, the current system suffers from many limitations, which curtail its usefulness as a research tool, and has never been useful as a Clinical Tool. The new system will have a dramatically enhanced performance: 1) decomposition time for typical contractions will be decreased from dozens of hours to a few minutes, 2) the automatic decomposition accuracy will be increased from 60 % to 95% - with provisions for assisted editing to reach 100% accuracy, 3) it will be able to decompose signals from dynamic as well as static contractions (which is a current limitation), 4) it will weigh less than 10 kg, and will have a notebook computer configuration, and 5) most importantly the decomposition algorithms will be completely rewritten using a newly developed knowledge-based Artificial Intelligence language blackboard platform developed by us. This platform has been used successfully to decompose polyphonic signals and radar spread spectrum signals having a complexity comparable to that of the EMG signal. The proposal is composed of 5 projects. The first and dominant project will be Design Driven. It describes the design and development of the new system, which has at its heart, a Knowledge-Based algorithms for decomposing the signals. The other four projects will be hypotheses-based and will address basic science questions and clinical applications that will reveal the utility of the new system. These projects will also be used to test and improve the evolving design of the new system. Project 2 will address the modifications, which occur in the firing of motor units as a function of Aging. Project 3 will address the phenomenon of motor unit substitution, which will be useful in Ergonomics work environments and in the Rehabilitation of patients with Peripheral Nerve Injury and Spinal Cord Injury. Projects 4 and 5 are two Clinical Studies. Project 4 will explore the use of quantified neuromotor activity for developing prognostic indicators for determining denervation and re-enervation of Paralyzed Laryngeal Muscles. Project 5 will study patients with acute ataxia following a Cerebellar Stroke to explore the manifestation of CNS disorders in the firing characteristics of the motoneurons.

STATUS OF RESEARCH AND PARTNERSHIP
Project # 1 – Decomposition of the EMG Signal – There are two main components to this project:
Software – The development of the software is on schedule. In FY4 we made further enhancements to the knowledge base of the system in order to improve its ability to resolve the errors arising from the initial signal processing. At this point, the system is able to automatically (i.e., without the need for manual editing) produce decompositions with an accuracy above 98% for complex isometric contractions involving 10-15 motor units whose action potentials undergo significant degrees of superposition. This is in contrast to around 60% accuracy obtained with other published EMG decomposition approaches. Furthermore, the latest version of our decomposition program has a run time of just a few minutes on a typical PC when analyzing complex isometric contractions lasting on the order of 1 minute. This program is now being used in the clinical investigations that are part of this BRP. To allow convenient inspection and editing of the decomposition results, we have developed a program with sophisticated graphical abilities that provides convenient access to

intermediate results of the decomposition program. This editing program has already been utilized in developing the knowledge base that led to the latest accuracy improvements in our decomposition software. It is our expectation that this program and its future refinements will also be of utility in the clinical investigations. Two papers are in preparation.

Hardware – The development of the system hardware is on schedule. The hardware consists of a Panel PC housing custom electronics used to acquire EMG signals detected with our existing needle electrodes. A laptop computer linked to the Panel PC contains software programs used to acquire, process, and display the collected data. The full compliment of four systems has been fabricated with two systems deployed for "beta site" testing, one with Dr. Roark at the New York Medical College, and a second with Dr. Manto at the Free University of Brussels, Belgium. The remaining two systems are undergoing data acquisition hardware and software evaluation at the NeuroMuscular Research Center.

Project # 2 – Aging – This project ended in FY3.

Project # 3 – Fatigue – The project is on schedule. In FY4 a mathematical model was developed to explain the observed behavior of the motor unit firing rates in the Vastus Lateralis (VL) muscle. In addition, the First Dorsal Interosseous (FDI) muscle was tested in an additional five subjects according to the fatigue-generating protocol used in the VL muscle experiments. Automatic decomposition of data from a total of 12 subjects using the new system has been completed. Detailed analysis of motor unit recruitment and firing rate at the beginning, middle, and end of the endurance time currently under way. We wish to compare the motor unit control properties of the two muscles. A paper was published.

Project #4 – Laryngeal Muscle Control – This project has evaluated ten subjects to date, with four additional subjects anticipated before the close of FY4 in accordance with the proposed schedule. Five of the subjects have been evaluated at the hospital bedside using the new data acquisition and analysis system. These experiences have provided helpful information to the partnership regarding applicability of the system to the important class of non-ambulatory patients within the hospital environment. The system has proven effective in providing clinical information not achievable by previous technologies within a time course to be useful for this patient class: paresis/paralysis of laryngeal muscles affecting swallowing, aspiration, respiration and speech. The information provided by the system helped to formulate treatment decisions for patients within a one hour time frame and offered critical guidance to surgical procedures subsequently undertaken on the same day of evaluation. As outlined in the proposal, clinical progress is being followed for all subjects (recovery, non-recovery, etc).

Project # 5 – Neurological Studies – This project is on schedule. Seven patients exhibiting an acute cerebellar syndrome following a stroke were studied with the new system. The FDI muscle of both hands was tested during a force tracking task similar to the one used in Project # 3. Prior to testing, each subject underwent a conventional neurological exam and MRI imaging of the brain. The EMG signals of all data sets, including several challenging cases of force tremor and target overshoot, were successfully processed by the enhanced decomposition algorithms. In addition, 13 patients with diffuse cerebellar atrophy, not previously proposed in the grant application, were evaluated. Recruitment threshold, firing rates and discharge variability of motor units are being analyzed with the intention to uncover differences between the two patient groups.

Partnership Collaboration – The partnership has matured well during the past four years. We have developed a comfortable bond of trust. We have continued our formal two-hour bi-weekly meetings with Dr. Nawab's group where we review progress and plan future activities. We are in continuous e-mail and telephone contact with our other two partners, Dr. Roark and Dr. Manto. We have continued our personnel exchange. Dr Roark visited the Boston labs three times in the past year, and Dr. Adam from Boston has twice participated in experiments at the New York clinical site and once at the hospital in Brussels. We held a two-day project review in Boston on March 8 and 9 and another on June 22. All partners were present. We will continue to exchange personnel during FY5 to facilitate the execution of the experiments and to review progress.

ISSUES: The collaboration amongst the BRP partners continues to progress smoothly and productively.

PI: Stephen P. DeWeerth, Professor
Wallace H. Coulter Department of Biomedical Engineering
Georgia Institute of Technology
Atlanta, GA 30332-0535
T: 404-894-4738
steve.deweerth@neuro.gatech.edu
http://www.neuro.gatech.edu/brp/

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

PARTNERS' NAMES AND AFFILIATIONS: Mark Allen (Georgia Tech), Greg Brewer (Southern Illinois University School of Medicine), Bruno Frazier (Georgia Tech), Ari Glezer (Georgia Tech), Michelle LaPlaca (Georgia Tech), Steve Potter (Georgia Tech), Bruce Wheeler (University of Illinois, Urbana/Champaign)

GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB) and 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.
Aims 1–3 represent the core elements of the technical development, and have been initiated in parallel during Years 1 and 2 of the grant. Aim 4 represents the culmination of the project through the combination of the prior aims, and is currently the focus of several research directions and technological refinements.

STATUS OF RESEARCH AND PARTNERSHIPS

The results to date from Year 2 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: Two primary techniques for micromachining the tower arrays are being developed: The first technique applies a novel double-exposure technique that produces towers and substrate from a common SU-8 deposition. This approach addresses column-to-substrate adhesion issues that have traditionally limited the aspect ratios achieved with conventional fabrication techniques. Two types of structures have been fabricated. The first is a relatively simple 'tower' structure, and is composed of high-aspect-ratio SU-8 pillars extending from either glass or silicon substrates. The second is similar, with the exception that multiple-level cross-bridges are suspended from tower to tower to provide additional horizontal surfaces for cells to adhere. Both fluidic channels and electrodes are being fabricated on these towers. The second technique applies surface micromachining technology to fabricate SU-8 based 2-D tower arrays flat on a planar surface. The 2-D tower arrays are released from the surface using

sacrificial layer technology and subsequently assembled into 3-D array configurations. SU8 tower arrays that contain both electrodes and hollow fluidic channels have been successfully fabricated. The tower arrays consist of either 8 or 16 towers with cross-bridges in one plane. Each tower (120 x 80 μm) contains two electrodes with corresponding insulated leads and one fluidic channel that runs along the entire length of the tower (70 x 25μm). For all towers, materials are detoxified and tested for cell viability and cell-material adhesion. The most effective treatment to detoxify SU-8 without causing damage to the SU-8, such as warping and cracking, was found to be a combination of heating at 155°C for 3 days, with 90J/cm^2 flood exposure, and 30 min of CO_2 supercritical extraction. Coating of SU-8 by paralyne, another polymeric material was also found to further reduce toxic leaching and thus improve viability of primary rat (E18) cortical neurons. To improve the neuronal adhesion properties of SU-8, the surfaces are treated, e.g. by oxygen plasma or coatings of adhesive polymers, and characterized by contact angle goniometry.

Specific Aim 2: We designed and built a microfluidic delivery system that consists of a 3D neural culture (perfusion) chamber centrally located on the top of a manifold to analyze the flow within the perfusion chamber induced by a multi-site injection/withdrawal circulatory system to provide uniform nutrient delivery and trophic support to cells. We use particle image velocimetry (PIV) to study the flow in the x-y planes along the height of the perfusion chamber. Jets are issuing normal to the field of view from an array of circular orifices that are 200um in diameter. We have tested the flow patterns through hydrogels, which will support cells that are distributed throughout the 3-D culture (as opposed to cultured directly onto the towers. The perfusion chamber was filled with SeaPrep agarose hydrogel (0.5% w/v). To analyze controllable, localized, nutrient delivery in the presence of hydrogel, we selectively imposed a concentration gradient induced by dye injection. The spreading penetration of the dye was clearly reduced due to increased pressure losses associated with transfer through small-scale pores.

Specific Aim 3: Our primary foci in this aim have been the completion of preamplifier technologies, and in the modeling of and design of circuitry for signal processing. Electromagnetic noise and stimulation artifacts cause difficulties when electronically recording from MEAs. We have developed models of the noise and artifacts to guide the design of electronics that scale well to thousands of electrodes. Our models predict that electrode impedance plays an important role in shaping incoming signals, determining thermal noise, and influencing the efficacy of stimulation. Our approach to optimally reducing thermal noise and improving the reliability of stimulation is twofold: minimize the impedance and match it across all electrodes. To this aim, we have fabricated a device that interfaces to the MEA and that facilitates the automated, impedance-controlled electroplating of micro-electrodes. Our model also gives an explanation for the stimulation artifact: the stimulation source charges the electrode capacitance through the low resistance, but that charge remains on the electrode after stimulation. The addition of a low impedance discharge pathway should decrease the stimulation artifact. We have designed integrated circuits that use multiple feedback pathways to discharge the electrode. In simulation, our circuits are able to eliminate the artifact.

Specific Aim 4: The focus of this aim over the past year has been on the specification and initial development of packaging technologies for the μNIS. At present we are developing both fluidic and electronic packaging technologies, which will be refined and applied to the system integration in Year 3.

ISSUES

The team of investigators brings together expertise from several disciplines and, as a result, have accomplished a significant number of the goals for Years 1 and 2 of the project. Despite having frequent interactions, including monthly video conferences, bi-annual retreats, a website, and an internal mailing list, an issue for this team is effective communication. Engineering culture is dictated by pushing the limit of current technology, while biologists strive to apply technology to answer scientific questions. This issue is evident in the current project, and is now beginning to be addressed as different aspects of the project evolve. For example, the biological component of the project requires cell-compatible materials that must withstand physiological conditions for an extended period of time. The microfabrication portion utilizes novel techniques and materials in order to make complex structures. These two approaches are not always compatible, and have presented challenges to the team. This issue is recognized by the group, however, and is benefiting the project, as well, by creating several possible avenues to accomplish the overall goal of developing 3-D microelectronic and microfluidic interfaces for neural cell culture. Coupled to this aspect of communication is the recognition and development of sub-projects and technologies. The investigators involved all train Ph.D. students, and anticipate that several new technologies and approaches will be used to answer neurobiological questions in novel and significant research projects. A goal for the remainder of the funding period is to foster this research and encourage teamwork and innovation.

PI: Marc A. Dichter, M.D., Ph.D.
University of Pennsylvania
Department of Neurology
HUP, 3 W. Gates
Philadelphia, PA 19072
T: 215 349-5166
Dichter@mail.med.upenn.edu

PROJECT TITLE: An Implantable Device to Predict and Prevent Seizures

PARTNERS' NAMES AND AFFILIATIONS:
Marc A. Dichter, M.D., Ph.D., Brian Litt, M.D. Peter Crino, M.D., Ph.D. Diego Contreras M.D., Ph.D., Leif Finkel, M.D Ph.D. (University of Pennsylvania); Doug Coulter, Ph.D. **(**Children's Hospital of Philadelphia); George Vachtsevanos, Ph.D. (Georgia Institute of Technology**)**

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

ABSTRACT
More than 25% of individuals with epilepsy cannot have their seizures controlled by current medical or surgical treatment. The need for new treatments is clear. We have assembled an ensemble of established investigators from the University of Pennsylvania, Georgia Institute of Technology, Children's Hospital of Philadelphia in a 5-10 year effort to create a novel therapy for refractory epilepsy: an implantable device capable of predicting epileptic seizures prior to electrical onset and triggering intervention to prevent their clinical expression. This complex task requires the focused efforts of a core of bioengineers from Penn and GIT in concert with experts in the fields of computer science, electrical engineering, clinical adult and pediatric epilepsy, neurophysiology, neuropharmacology and molecular and cellular neuroscience. This research partnership has three major thrusts: (1) Seizure Prediction: Developing and refining algorithms capable of predicting seizures hours to minutes prior to electrical and clinical onset. These algorithms are based upon signals obtained from implanted biosensors in adults, children and animal models of human epilepsy, (2) Mechanisms of Ictogenesis: Unraveling the neurophysiologic, neuronal network, cellular and molecular, mechanisms underlying the preictal (preseizure) changes identified by these algorithms through in-vitro and in-vivo investigations in the laboratory and clinical settings. Experimental observations will be incorporated into computer simulations of these mechanisms to facilitate development of better prediction and intervention strategies, (3) Therapeutics: Developing interventions aimed at specific points in the "ictogenic" process based on electrical brain stimulation to disrupt the cascade of events leading to seizures while preserving normal brain function.

STATUS OF RESEARCH AND PARTNERSHIP:

Seizure Prediction, Seizure Precursors and Algorithm Development
In the third year of this project we continued to make great progress in four areas: (1) Algorithm development: refining methods for detecting seizure precursors and identifying periods of increased probability of seizure onset. This work has been extended to neocortical epilepsy, and we have new exciting findings linking high frequency and unit activity to seizure generation, (2) application of engineering principles to brain stimulation to pre-empt and abort seizures, including new methods using machine learning, such as support vector machines, which continuously learn from patient seizures and stimulation trials and (3) using these tools to gain more insight into mechanisms underlying seizure generation. Finally, (4) we have realized the first major goal of this project, translating our work into a first generation implantable clinical device for treating epilepsy. Our licensee, ***NeuroPace, Inc.,*** is currently enrolling patients in a multi-center, prospective, blinded, controlled clinical trial of a responsive, implanted brain stimulation device to predict/ detect seizures and stimulate to prevent clinical symptoms, based upon algorithms licensed from UPenn and Georgia Tech, developed under this grant.

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

Circuit and cellular biophysical mechanisms during generation of the preictal cascade
We have spent the past year examining how seizures initiate in the limbic system, with a particular focus on factors regulating seizure entry into the hippocampus. To conduct this circuit level analysis with sufficient spatial and temporal resolution, we have used voltage sensitive dye imaging (VSD) techniques, and a very fast 80X80 CCD camera, which allows us to sample activity at frequencies of 1-5 kHz and simultaneous patch clamp and field potential recordings. Using these VSD recordings, we have determined that the dentate gyrus acts as a filter, determined primarily by feedforward/ back GABAergic inhibition, regulating entry of information and seizure activity into the hippocampus.

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

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).

Seizure suppression by brain stimulation in animals with seizures
We initially attempted to develop methods to suppress acute seizures originating in the hippocampus evoked by repetitive electrical stimulation, but no stimulus pattern was able to abort the ictogenesis. We are now focusing on seizures induced by focal applications of convulsants to neocortex and hippocampus to determine if these acute seizures can be prevented by direct brain stimulation. This acute work is proceeding in parallel with stimulation to prevent seizures in freely moving, spontaneously seizing animals implanted with intracranial electrodes.

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

PI: Johnathan Dordick, Ph.D.
Rensselaer Polytechnic Institute
Chemical and Biological Engineering
129 Ricketts
Troy, NY 12180
T: 518-276-2899
F: 518-276-2207
dordick@rpi.edu
http://enzymes.che.rpi.edu

PROJECT TITLE: High-Throughput Solid-Phase Combinatorial Biocatalysis

PARTNERS' NAMES AND AFFILIATIONS:
Douglas S. Clark, Ph.D. (University of California, Berkeley)
Jeffrey A. Reimer, Ph.D. (University of California, Berkeley)
Shekhar Garde, Ph.D. (Rensselaer Polytechnic Institute)
Alexander M. Klibanov, Ph.D. (Massachusetts Institute of Technology
Brian H. Davison, Ph.D. (Oak Ridge National Laboratory)

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

ABSTRACT
Rapid developments in genomics, proteomics, and combinatorial chemistry have reshaped the field of drug discovery, providing new drug targets for selective screens and new compounds to be tested in those screens. While combinatorial methods have given rise to large libraries of compounds, typically these compounds result in improved lead candidates that must undergo further transformations by conventional medicinal chemistry to yield new drug candidates. Bioengineering, in the context of high-throughput combinatorial methodologies, has not impacted lead optimization nearly as much as it has lead discovery, mainly because of the highly selective, intricate chemistries often required to optimize lead compounds and the lack of a suitably broad high-throughput platform. Combinatorial biocatalysis can help overcome these obstacles by exploiting the exquisite selectivity and unique reactivity of enzymes and microbial biocatalysts; however, to date this technology has been limited to the derivatization of soluble substrates. We propose to expand 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. The specific aims are:
1. To expand the breadth of biocatalysis on solid- and polymer-supported compounds in aqueous and nonaqueous media; 2. To develop strategies for attaching lead compounds and removing their derivatives from solid and polymeric supports; 3. To demonstrate high-throughput, combinatorial biocatalytic lead optimization of complex natural and synthetic molecules, screen resulting derivatives for biological activity, and scale up structurally and functionally interesting derivatives using biotransformations
Successful completion of this research program will result in a powerful methodology that can be used by biomedical investigators in the search for new, more potent small molecule therapeutics.

STATUS OF RESEARCH AND PARTNERSHIP
The emergence of combinatorial methods for new compound synthesis and drug discovery has led to a dramatic increase in the number of drug candidates for *in vitro* and ultimately *in vivo* testing. Nevertheless, the clinical progression of new chemical entities to pharmaceuticals remains hindered by the relatively slow pace of technology development in increasing the throughput of lead compound optimization in the preclinical phase of drug development. For this reason, our Partnership, which was funded in February 2004, has begun to develop the fundamental enzymology and specific technology components needed for solid-phase combinatorial biocatalysis. This technology is an iterative approach

that exploits the unique properties of biocatalysts to derivatize lead compounds of virtually any size and produce libraries of structurally diverse compounds that are well-suited as preclinical candidates.
In our first three months of support, we have begun to expand 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. This work is collaborative between the Dordick and Clark laboratories and includes enzymatic derivatization of surface-bound lead compounds. In one example, phenols are bound to amino-functionalized glass slides and to Tentagel resins that contain a seed phenol. Peroxidase is then used to catalyze the selective and sequential condensation of natural phenols in a spatially-addressable format on the slides. The oligophenols generated using this approach range from dimers to pentamers, and detailed structural information has been obtained. Quinone- and nonquinone products are generated and are now being screened as phenolic-based inhibitors of NADPH oxidase with an impact on inflammatory disease therapies. In related work, the kinetics of enzymatic catalysis on solid-phase substrates is being investigated to identify optimal supports for continued work. Controlled pore glass is being used as a solid support for bergenin (as a model natural product lead) derivatization. Two coupling chemistries have proven to be especially attractive. The first involves coupling bergenin directly to a carboxylic acid support, or first coupling bergenin to Boc-protected phenylalanine in solution, followed by coupling of the free amine on the phenylalanine-bergenin compound to the acidic support. Coupling yields of 50-60% have been achieved. The second chemistry involves coupling of bergenin enzymatically acylated with a divinyl adipate moiety onto an amine support, either directly (ester-to-amine coupling) or through a phenylalanine linker. Thus far, 30% yields have been obtained for this coupling chemistry. Current efforts have been focused on demonstrating the viability of the remaining attachment chemistries (both directly and with the phenylalanine linker) in the solid phase, as well as on optimizing strategies for removing the substrate from the support. At this point, non-enzymatic strategies are primarily being pursued. Once these have been sufficiently developed, we will focus on enzymatically cleaving the linker from the support.
In parallel (at all institutions), we are studying the key factors that will enable enzymes to function on solid-supported lead compounds, including the structure and function of enzymes solubilized in nonaqueous media. In one example, we have developed a novel ion-pairing method for solubilizing enzymes in organic media, which will be needed to perform acylation reactions on solid-supported leads. Termed “direct solubilization,” this method yields both a more efficient solubilization and a more active enzyme than other solubilization techniques. We have begun a more detailed mechanistic study that is focused on the effects of solvent polarity on enzyme activity in non-aqueous media. In a second example, we are examining how the reaction conditions influence enzyme specificity, a critical variable in the use of combinatorial biocatalysis for lead optimization. Using peroxidase as a model enzyme, the enantioselectivity of alkyl sulfide sulfoxidation has been found to be influenced by the polarity of the organic solvent. Using structure-activity relationships, we suggest that the stereochemistry of the carbon atom immediately adjacent to the sulfur atom dramatically affects the latter’s HRP-catalyzed sulfoxidation.
Specific areas of research under development include the expansion of enzymatic reactions on solid- and soluble polymer-supports containing model and natural product leads, the preparation of organic solvent stable (and as necessary soluble) enzyme formulations with high activity and controlled selectivity, and the spectroscopic and computational analysis of these enzyme preparations to provide a mechanistic and theoretical framework for understanding enzymatic catalysis in these synthetically relevant environments. Each of these research directions sets the stage for the more complete development of high-throughput solid-phase combinatorial biocatalysis as a new tool in drug development.

ISSUES
There are no issues of major concern. The two academic subcontracts have been put in place and are proceeding smoothly. Minor problems with the national lab subcontract are being addressed. The interaction of four geographically separated partners is running efficiently.

PI: Mark Doyle, Ph.D.
Allegheny General Hospital
320 East North Avenue
Pittsburgh, PA, 15212-4774
T: 412 359 4243
Fax 412 359 8964
mdoyle@wpahs.org

PROJECT TITLE: Rapid Flow Evaluation by Magnetic Resonance Imaging

PARTNERS' NAMES AND AFFILIATIONS:
Andreas Anayiotos, Ph.D. (UAB Bioengineering, Birmingham, AL);
Eduardo Kortright, Ph.D. (UNO, Computer Science, LA);
Robert W.W. Biederman M.D. (AGH, Cardiology, Pittsburgh, PA)

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 aortic valvular diseases. Currently, aortic valve diseases are primarily assessed using echocardiography which is widely available, but nevertheless has several important limitations in characterizing flow fields, including views are restricted by the availability of appropriate acoustic windows, results are operator dependant, velocity is detected in only one direction relative to the probe and that primarily 2D views are used to characterize a 3D flow field.
While MR VEC imaging has the potential to provide more comprehensive flow field data than does echocardiography, clinical application of MR VEC imaging has been hampered by its relatively long acquisition times. The powerful gradient systems now available on MRI scanners allow high quality cardiac cine scans to be acquired in comfortable breath-hold times. However, the scan time required for VEC imaging with velocities resolved in 3D is still prohibitively long for most clinical applications.
The goal of this proposal is to implement a rapid MRI approach that has potential to accomplish VEC imaging in a conventional breath-hold time. Development includes MR scanner sequences modification, determining its limits of applicability using computer modeling of flow fields and testing using flow models. In parallel with implementation and validation of the acquisition sequence, processing tools will be developed to analyze the time resolved 3D flow field data sets. Following the development stage, clinical application will be made to patients with aortic valvular diseases.

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

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

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. For instance, the BRISK approach allows rapid breath-held cine acquisitions to be acquired. The initial emphasis with this research was to increase the scan speed. However, while BRISK could produce cine series that were comparable to conventional cine series, we became more acutely aware of scan quality issues, especially when visualizing features associated with the apparatus of flow such as the leaflets of cardiac valves. Consequently, we implemented BRISK cine imaging to acquire images in the same time as conventional cine scans, but using the BRISK properties to improve visualization of rapidly moving features. By this means we were able to improve visualization of the valve leaflets, which ultimately will lead to improved positioning of the control volume. Another aspect of the project that was enhanced by the cross-disciplinary nature of the investigation was the appreciation for features other than scan speed that affect accuracy of flow data. In the literature, slice orientation relative to flow is usually discussed in terms of scan efficiency. Our simulations in this area showed that accuracy and efficiency varied dramatically depending on slice orientation. Also, for any given slice orientation series, computation of the associated control volume surface area should take in to account the exact nature of the intersecting slices of an acquisition series. Further, the representation of the typically much lower in-plane flow features in complex flowfields has traditionally been problematic for MRI VEC data. We have analysis to suggest that this is not merely a problem associated with dynamic range of the data. Investigations are underway to analyze this data to make BRISK and its variants more sensitive to these features. In summary, we are very encouraged by the partnership and believe that its very structure has contributed to this research project.

PI: James Duncan, PH.D.
Yale University
Diagnostic Radiology, Biomedical Engineering and Electrical Engineering
333 Cedar Street
New Haven, CT 06520-8042
T: 203-785-6322
F: 203-737-4273
james.duncan@yale.edu
http://ipagwww.med.yale.edu

PROJECT TITLE: Bioimaging and Intervention in Neocortical Epilepsy

PARTNERS' NAMES AND AFFILIATIONS:
James Duncan/Lawrence Staib (Yale University), Douglas Rothman/Robin De Graaf (Yale), Hoby Hetherington (Albert Einstein), Todd Constable (Yale), Dennis Spencer (Yale), Thomas Vaughan (University of Minnesota), Rainer Birkenbach (BrainLAB AG)

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 3 academic institutions (Yale (lead institution), Albert Einstein and the University of Minnesota) and industrial partner (BrainLAB, AG) to carry out four integrated programs of scientific investigation and bioengineering development in the area of bioimaging and intervention: 1) development of high resolution fMRI and MRS at 4T for the study of epilepsy; 2) investigation with MRS of the relationship between neuronal damage or loss through the measurement of N-acetyl aspartate (NAA), alterations in neurotransmitter metabolism through the measurement of gamma amino butyric acid (GABA) and glutamate, and abnormalities in electrical activity in the epileptogenic region and surrounding tissue; 3) investigation of the relationship between fMRI activation amplitude and the cognitive task, underlying cortical structure, cortical metabolic state, and physiology, and the impact of epilepsy on these factors; 4) development of integration methodologies for fusing multimodal structural and functional (image- and electrode-derived) information for the study and treatment of epilepsy.

STATUS OF RESEARCH AND PARTNERSHIP
Our efforts are proceeding as planned. We have made significant progress in a number of areas.

1. With respect to 4T coil development and dynamic shimming, during the past year we have completed the fabrication and testing of an actively detunable TEM volume transmit/receive coil with two actively detunable four channel phased arrays. The latter set has provided a 4-6 fold increase in SNR over that of a

conventional head volume TEM RF coil while providing an effective sampling depth of up to 6cm. In addition, our Dynamic Shift Updating strategy has now been implemented on the 4T human system at Yale and has yielded quite promising results.

2. In MRS, we have focused on developing our NAA MRS imaging and analysis strategies related to imaging neuronal loss and damage using a conventional coil configuration (four overlapping coils). We have acquired spectroscopic images of human brain NAA with 125μl voxels with a SNR of 10-12.5:1. Using the volume coil as both a transmit and receive coil with the phased array in place has provided the ability to acquire whole brain anatomical images for localization without the need for hardware changes or patient repositioning, and the ability to obtain amplitude and phase correction factors for the phased array reconstructions using conventional low contrast water images. We have used this coil to acquire spectroscopic images from patients with neocortical epilepsy (5) and controls (7).

3. In our ongoing efforts to develop fMRI methods, in the past year we have made progress in the acquisition of field map data for use in distortion correction from our high field human systems and are in the process of using this data for distortion correction of fMRI time-series data acquired from human control subjects. This approach is instrumental in permitting accurate fMRI registration to the high resolution anatomical image datasets. In addition, we have begun to acquire fMRI time series data on our high field human magnets in order to begin to compare these data to the MRS data described above in the coming year.

4. We have made significant progress in the development of integration methods and our image analysis platform. In order to improve the reconstruction accuracy of the brain surface stereo algorithm, we are incorporating surface blood vessel features in a probabilistic formulation. Initial testing on gel phantoms has shown promising improvement. We have also included proper boundary condition modeling which has the promise of yielding improved brain shift compensation in deeper structures.

We have implemented methods to localize implanted electrodes as defined from their 3D coordinates in registered CT and MR images. This capability has become part of the clinical routine for these patients (8 patients have been processed) and facilitates the comparison of electrode recordings with our pre-operative MR-derived measurements. We have performed initial experiments to relate MRS imaging measurements of NAA to electrophysiologic data revealing a spatial correlation linking abnormal electrical spiking (higher spike counts) and abnormal NAA levels (lower NAA/creatine ratios) in epileptogenic regions.

A fully featured Vector Vision system from Brainlab (identical to the one in the neurosurgery operating room), including the research interface, has been installed in the Yale image analysis laboratory for methods development and testing.

ISSUES:
All partners have been communicating effectively. Transfer of equipment, software and data has proceeded smoothly.

PI: **EATON, GARETH R., PH.D.**
Department of Chemistry and Biochemistry
University of Denver
2101 E. Wesley Ave.
Denver, CO 80208-2435
T: 303-871-2980
F: 303-871-2254
Email: geaton@du.edu

PROJECT TITLE: In Vivo EPR Bioengineering Research Partnership

PARTNERS NAMES AND AFFILIATIONS:
Gareth R. Eaton, Ph.D. , Department of Chemistry and Biochemistry, University of Denver
Arthur H. Heiss, Ph.D., Bruker BioSpin, EPR Division, Billerica, MA

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

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

STATUS OF RESEARCH AND PARTNERSHIP
In the past year we have made substantial progress in both instrumentation and data analysis. Lithium phthalocyanine (LiPc) and Nycomed triarylmethyl radical (trityl-CD_3) have been proposed as probes for in vivo oximetry. EPR spectra at 250 MHz for a single crystal of LiPc in the absence of oxygen and for a deoxygenated aqueous solution of trityl-CD_3 were obtained at scan rates between $1.3x10^3$ G/s and $3.4x10^5$ G/s. These scan rates are rapid relative to the reciprocals of the electron spin relaxation times (LiPc: T_1 = 3.5 μs and T_2 = 2.5 μs; trityl: T_1 = 12 μs and T_2 = 11.5 μs) and cause characteristic oscillations in the direct-detected absorption spectra. For a given scan rate, shorter values of T_2 and increased inhomogeneous broadening cause less deep oscillations that damp out more quickly than for longer T_2. There is excellent agreement between experimental and calculated line shapes and signal amplitudes as a function of radiofrequency magnetic field (B_1) and scan rate. When B_1 is adjusted for maximum signal amplitude as a function of scan rate, signal intensity for constant number of scans is enhanced by up to a factor of three relative to slow scans. The number of scans that can be averaged in a defined period of time is proportional to the scan rate, which further enhances signal amplitude per unit time. Longer relaxation times cause the maximum signal intensity to occur at slower scan rates.

The initial experiments were performed with sinusoidal field sweeps because for modest sweep widths these are available in commercial instrumentation, which facilitated the initial experiments.

However, in the sinusoidal sweeps the scan rate changes continuously across the sweep. We have now constructed a system that generates triangular sweeps with sweep frequencies between 1 and 10 kHz and sweep widths of 1 to 10 G and good sweep linearly over approximately 90% of the sweep range. In these sweeps the scan rate is constant across the spectrum, which facilitates deconvolution of the rapid scan response and recovery of the undistorted signal.
A major advantage of the rapid scan approach is that it provides the absorption signal directly, without need for signal integration. The signal-to-noise in the absorption signal decreases linearly with gradient, whereas the signal-to-noise for a traditional first-derivative EPR signal decreases quadratically with gradient. We have obtained preliminary 2-D images and work is underway on the hardware and software for 3-D images.
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.

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

PI: V. Reggie Edgerton, Ph.D.
UCLA Department of Physiological Science and Department of Neurobiology
621 Charles E. Young Drive LS 1804 Box 951527
Los Angeles, CA 90095-1527
T: 310-825-1910
F: 310-267-2071
vre@ucla.edu

PROJECT TITLE: Robotically Generated Locomotion in Rodents

PARTNERS' NAMES AND AFFILIATIONS: Dr. Reggie Edgerton (Department of Physiological Science, UCLA), Dr. Ray de Leon (Department of Kinesiology and Nutritional Science, Cal State LA), Dr. David Reinkensmeyer (Department of Mechanical and Aerospace Engineering, UC Irvine)

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

ABSTRACT
The adult mammalian lumbar spinal cord can learn to step in the absence of descending input from the brain. The ability of the spinal cord to learn is an extremely important finding for tens of thousands of spinal cord injured patients, as it could mean the difference between being confined to a wheelchair or being able to stand and take some steps. Understanding how to teach the spinal cord to step through effective rehabilitative training has immediate clinical application in itself and can also play a crucial role in enhancing the efficacy of other potential therapeutic interventions for spinal cord injuries. One method of rehabilitative training, i.e. body weight supported locomotion on a treadmill, has been successful in enhancing locomotor recovery in spinal cord injured animals. There is growing evidence that this form of training can also be used to improve walking in humans that have suffered a stroke or spinal cord injury. The success of training, however, depends on the generation of appropriate patterns of sensory information during weight bearing stepping. We have developed a robotic system to train the hindlimbs of spinally transected rodents to step on a treadmill. The robotic system provides precise control of forces acting on the hindlimbs during stepping and also provides on-line measurement of step cycle trajectory characteristics so that locomotor performance can be quantified quickly and objectively. We hypothesize that the recovery of hindlimb stepping in spinally transcted rats will be enhanced by robotic-controlled locomotor training. We will use the robotic system to control critical training parameters such as the amount of weight bearing on the hindlimbs, the coordination of movements between the two hindlimbs and the amount of assistance provided during training. The first two aims examine the question of whether providing a maximum amount of weight bearing on the hindlimbs is the most effective weight bearing pattern during training. We will program the robotic device to 1) slowly increase the amount of hindlimb weight bearing within a stepping episode and 2) apply a force field that increases loading by exerting a downward force on the hindpaw. The third aim will examine the effects of imposing different hindlimb coordination patterns on the recovery of stepping. The robotic system will be programmed to train either an alternating gait pattern or an in-phase gait pattern in the hindlimbs. Finally, the fourth aim is to examine the extent that mechanical assistance during training should be provided to facilitate learning by the spinal cord. The recovery of stepping will be compared in rats that receive constant, robotic assistance throughout the step cycle versus rats that receive robotic assistance on an “as-needed” basis. The results of this project will provide a needed behavioral foundation for future research that identifies the specific neurophysiological and molecular mechanisms underlying sensory-enhanced spinal learning. These data will also provide insight into development of body weight support control and manual (or robotic) intervention for human locomotor training.

STATUS OF RESEARCH AND PARTNERSHIP

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

ISSUES

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

PI: Emery N. Brown, M.D., Ph.D.
Massachusetts General Hospital
55 Fruit St.
Boston, MA 02114

PROJECT TITLE: Dynamic Signal Processing Analyes of Neural Plasticity

PARNERS' NAMES AND AFFILIATIONS:
Wendy A. Suzuki, Ph.D., Matthew A. Wilson, Ph.D.

GRANTING NIH INSTITUTE/CENTER: National Institute on Drug Abuse

ABSTRACT
The area of bioengineering research will be the development of neural signal processing algorithms by combining the theory of point processes and adaptive estimation to study neural plasticity during learning and memory formation. The experimental investigations will 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 designs new methods for neural signal processing appropriate for dynamic analysis of multiple simultaneously recorded neural spike trains. The algorithms we design 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 long-term objectives of this partnership are: to establish a combined experimental-signal processing approach to characterizing neural plasticity and how it relates to learning, memory formation and behavior; to develop broadly applicable signal processing tools for analyzing the dynamic behavior of neural ensembles; and to establish an interdisciplinary research environment so that undergraduates, graduate students and post-doctoral fellows can be well trained in both the cutting edge experimental methods and signal processing techniques that are jointly required to study a complex question such as the dynamics of neural information encoding in the brain. The health implications of this work are a more fundamental understanding of neural plasticity and, as a consequence, how it affects normal physiological processes such as growth, development and learning as well as pathologic conditions such as drug addiction, Alzheimer's disease and chronic pain. More accurate quantitative characterizations of neural information dynamics coupled with improved signal processing algorithms may also lead to innovative approaches to creating machine-brain interfaces and designing neural prosthetic devices.

STATUS OF RESEARCH AND PARTNERSHIP
Specific Aim 1: Dynamic Analysis of Information Encoding within the Hippocampus (Matthew A. Wilson, MIT). Analysis of receptive field dynamics and shape characteristics on the PSD-95 mutant line has been completed and the results are being submitted for publication. Behavioral and electrophysiological characterization of the DG-specific NMDAR-KO animals has continued with initial preliminary results indicating specific deficits on the new task developed for this purpose. Corresponding electrophysiological work has also begun on the CA3-restricted KO mice. Publications have also resulted from collaborative work under Project 3 (Barbieri et al., Neural Computation, 2004). Collaborative analyses of multiple single unit data collected under Project 1 relating to hippocampal sleep reactivation have been initiated using novel algorithms developed under Project 3.
Specific Aim 2: Dynamic Analysis of Information Encoding Within the Hippocampus and Adjacent Regions of the Medial Temporal Lobe (Wendy Suzuki, NYU). Important progress has been made in two different areas. First, substantial data has been gathered during encoding of new associative memories in the perirhinal cortex (90 cells in 2 animals). We are now able to compare in great detail the patterns of activity in these memory-related areas to our previous findings in the adjacent hippocampus (Wirth et al., 2003). We find that similar portions of cells in both areas respond selectively to the learning task (location-scene association task) and both areas signal learning with dramatic changes in neural activity (termed changing cells). The

major difference between the two areas is the timing with which the change takes place. The hippocampus appears to change significantly earlier relative to learning compared to the perirhinal cortex, suggesting that the hippocampus may play a more dominant role in early associative learning compared to the perirhinal cortex. The second important new finding concerns the role of the hippocampus and perirhinal cortex in signaling very well-learned information. We recently showed that the hippocampus signals well-learned information with a significantly more selective response to well-learned stimuli compared to novel stimuli both in the stimulus presentation period as well as during the immediately following delay period (Yanike et al., 2004a). In contrast, while perirhinal cells respond to well-learned stimuli with a significantly more selective response compared to novel stimuli during the scene period of the task, the signal is not seen during the delay period of the task. Thus, these findings show that while both the hippocampus and perirhinal cortex signal well-learned information during stimulus presentation, only the hippocampus carries this information over into the delay period (Yanike and Suzuki, 2004b).

Specific Aim 3: Dynamic Signal Processing Methods for the Analysis of Neural Plasticity (Brown, MGH/HMS; Solo, University of Michigan). Important progress has been made in several areas. We have developed a general paradigm for point process adaptive filtering (Eden et al., 2004). This manuscript extends our previous work and defines for point process observations, the analogs of the steepest descent recursive least squares and the Kalman filter algorithms for continuous-valued processes. This work significantly enhances our ability to track neural plasticity on a millisecond time-scale. We have also presented a general paradigm for decoding using ensemble neural spiking activity in the state space framework (Barbieri et al., 2004). This work makes it possible to use an arbitrary point process model in the decoding algorithm. It also makes clear the relation between information theory and neural spike train decoding. This has been a major collaborative effort with Project 1. We have developed an efficient algorithm for fitting multivariate point process models of neural spiking activity (Okatan et al. 2004). This algorithm makes it possible to estimate simultaneously the effects of members of a group of neurons on each other. We have also introduced a new paradigm for characterizing learning from binary responses in behavioral studies using the state-space framework to define learning as a dynamic process (Smith et al. 2004). We presented new definitions of the learning trial and show that our approach characterizes learning more accurately than current methods. These algorithms are available on our website for use by neuroscientists. This was a major collaboration with Project 2. We have published a review of the theory of point processes for neural systems (Brown, 2004) and a detailed review of the state-of-the art of analysis methods for multiple neural spike trains (Brown, Kass, Mitra, 2004). This work will help neuroscientist understand what are the important research questions.

Significance:

Specific Aim 1 Through the coordinated development of new spatial behavioral tasks, targeted genetic manipulations in hippocampal subregions, and the simultaneous use of multiple single unit recording in rodent model systems, we have begun to establish the means to directly link receptive field properties and dynamics to behavioral performance on a trial by trial basis, similar to methods employed in primate learning and memory studies.

Specific Aim 2 The findings related to Specific Aim 2 are now allowing us to compare in a detailed way the dynamic patterns of activity observed during new associative learning between the hippocampus entorhinal and perirhinal cortex. These findings not only have important implications for understanding the computations these two areas perform during learning, but provide important data for comparison with computational models of these areas during learning.

Specific Aim 3 The findings related to Specific Aim 3 are now allowing us to track more accurately neural plasticity in single neurons and in groups of neurons. It also allows us to characterize accurately behavioral changes in learning experiments and to give a more accurate statement of when learning occurs in these studies. We will apply these methods in the analysis of the neural and behavior data from Projects 1 and 2.

Plans

Our plan is to pursue the specific aims as stated in the original application. We will continue hiring required personnel.

ISSUES

None

PI: Michael S. Feld
Massachusetts Institute of Technology
G.R. Harrison Spectroscopy Laboratory
77 Massachusetts Avenue; 6-014
Cambridge, MA 02139
T: 617-253-7700
F: 617-253-4513
msfeld@mit.edu
http://web.mit.edu/brp/ http://web.mit.edu/physics/facultyandstaff/faculty/michael_feld.html

PROJECT TITLE: Spectroscopic imaging and diagnosis of neoplasia

PARTNERS' NAMES AND AFFILIATIONS:
Vadim Backman (Northwestern University, Evanston, IL); Kamran Badizadegan (Massachusetts General Hospital, Boston, MA); Christopher Crum (Brigham and Women's Hospital, Boston, MA); Sarah Feldman (Brigham and Women's Hospital); Irene Georgakoudi (Massachusetts General Hospital); Karl Munger (Harvard Medical School, Boston, MA); Stanley Shapshay (Boston Medical Center, Boston, MA).

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 chemopreventive and imunotherapeutic agents. 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 histologic 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 six different institutions with expertise in optics, medicine and 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 two 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 LSS imaging instruments for use in two separate clinical projects. Over the past year, we have built and tested two optical probe instruments for use in the clinical projects. The instruments are fully operational and are being used routinely. A breadboard clinical imaging instrument has also been built and is currently under further development in the laboratory. In addition, we have developed new software for real-time instrument control and automated data analysis for the optical probe instruments.

Development of Novel Spectroscopic Methodologies. The goals of this study are (1) to establish how LSS imaging of multilayered tissues is affected by multiple light scattering, and (2) to extend the capabilities of LSS to obtain complimentary information about tissue structure using multiple light scattering. We have accomplished our first-year goal of developing a specialized light scattering instrument capable of collecting polarization dependent spectral, angular, and spatial information about light scattering by multilayered tissues. The instrument is currently being used to collect and analyze tissue phantom data under various scattering and absorption conditions in the laboratory.

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 year 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 initial studies show expected changes in spectroscopic markers of cell lines after exposure to oncoproteins and after induction of apoptosis.

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, data from 35 patients with cervical lesions and 53 patients with oral lesions have been collected using FastEEM optical probe instruments. These data are at various stages of analysis and correlation with pathological diagnoses, tissue morphometric parameters and HPV status.

ISSUES

The rate of patient accrual for clinical studies in the first year has been less than projected. Unexpected changes in key personnel that were largely responsible for this issue have been resolved, and additional clinical centers have been added to accelerate the rate of patient accrual in the future.

PI: Dr. John A. Frangos
La Jolla Bioengineering Institute
505 Coast Boulevard South
La Jolla, CA 92037
T: 858-456-7505
F: 858-456-7540
frangos@ljbi.org
http://www.ljbi.org

PROJECT TITLE: Anti-Inflammatory Coatings for Biomaterials

PARTNERS
Prof. Marcos Intaglietta
Department of Bioengineering
University of California-San Diego

Prof. Joanna McKittrick
Materials Science Program
University of California-San Diego

Prof. Lars Bjursten
La Jolla Bioengineering Institute

Dr. Christine Orme
Lawrence Livermore National Labs

Dr. Steven Hurson
Nobel Biocare USA

GRANTING INSTITUTE: 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), wound dressing material with anti-inflammatory properties (3M), and dental materials with improved osteointegration (Nobel Biocare). Our overall objective is to provide the proof-of-principle to our industrial partners, which will encourage them to participate in more specific product development.

STATUS OF RESEARCH AND PARTNERSHIP

Project 1: The mechanisms of action of titanium coatings on the inflammatory process have been investigated. Using AFM and Raman spectroscopy, we have evidence that titanium peroxy gel is readily formed. This may represent one mechanism for the anti-oxidant characteristics of titanium.

Project 2: We have succeeded in producing coatings of anatase crystalline isoforms of titanium dioxide on surfaces at room temperatures. These coatings are well-adhered to quartz and silicone elastomer.

Project 3: We have tested the new surfaces for their ability to mitigate the production of reactive oxygen species by human neutrophils and murine macrophages. The results are encouraging. We are preparing a manuscript.

Project 4: Mechanical mismatch seems to be of major importance for the development of foreign body response at implanted biomaterials. All currently used materials are much stiffer than the tissue they are implanted into with the exception of bone tissue. A series of hydrogels with varying degrees of crosslinking and elastic modulus have been implanted in muscle tissue. When the modulus approaches that of the tissue (5-20 kPa) the foreign body response is virtually eliminated. Currently the studies are expanded to include a series of silicon elastomers and materials with a pliable coating on top of a stiffer base material. Further, an in vitro experimental system has been developed based on an AR-instrument rheometer that allows us to apply minute and controlled cyclic stresses to macrophages on materials interfaces. Preliminary data shows that cell necrosis as well as gene expression is closely linked to the shear applied to the cells.

Project 5: We have explored collaborations with new industrial partners. There have been discussions with three companies about our anti-oxidant ceramic coating technology for biomaterials: Medtronics, Philometron and Hewlett-Packard.

ISSUES

None.

PI: Jeffrey Fredberg
Harvard School of Public Health,
665 Huntington Ave
Boston, MA 02115
T: 617 432 0198
T: 617 432 3468
jfredber@hsph.harvard.edu
http://www.hsph.harvard.edu/physiology/

PROJECT TITLE: Micromechanics of airway smooth muscle cells in culture

PARTNERS:
Harvard University (Boston)
Universitat Biofisica y Bioenginyeria, Univ. (Barcelona, Spain)
Dalhousie Univ. (Halifax, Nova Scotia, Canada)
University of Erlangen-Nuremberg (Germany).

GRANTING INSTITUTE: NHLBI

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-discliplinary 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
This partnership was comprised initially of three institutions (Harvard University, University of Barcelona, Dalhousie University), but due to the move last year of Dr. Fabry from Harvard to the University of Erlangen, Germany, that university now represents a fourth partnering institution within our international consortium. Together, we have developed into a cohesive interdisciplinary research team that is highly productive and highly interactive, as shown by the fact of 31 peer-reviewed publications in the current grant period, 22 of which were coauthored by two or more consortium partners, 5 of which were invited reviews or perspective articles, and all of which appeared in leading journals of our respective fields including AJP:Cell Physiol., Biophysical J., Physical Review Letters, PNAS, and NEJM. We had proposed a multidisciplinary design-directed bioengineering project. Our goal was to develop a micromechanical technology to measure rheological properties of the single adherent living airway smooth muscle (ASM) cell in culture. We proposed also to measure the time-course of property changes in response to contractile stimuli or genetic manipulations of cytoskeletal (CSK) scaffolding proteins. Simply put, we sought to develop a technology to study the airway smooth muscle cell in culture conditions (as regards biochemical assays not at all new), but with accessible mechanical endpoints (altogether new), as it were, providing a model in culture of asthmatic bronchospasm. Insofar as muscle contraction and bronchospasm are quintessential mechanical events, the absence of an everyday mechanical assay amongst our research tools represented a telling blind spot. To fill this gap we proposed to advance a technology called magnetic twisting cytometry (MTC). Ferrimagnetic microbeads are ligand-coated and then bound to integrin receptors at the cell surface. The resulting complex (bead/receptors/focal adhesions) becomes tightly connected to stress-bearing cytoskeletal structures and the contractile apparatus. By imposing a uniform magnetic field upon magnetized beads, a very small torque is applied to each bead, and resulting bead motions deform structures deep in the cell interior.

Thus, the technology becomes in effect a micro-rheometry system that can probe – in cell culture conditions and over a wide range of time scales – contractile responses and underlying cellular rate processes.

Each of our 5 specific aims was completed successfully, but technical accomplishments greatly exceeded expectations. We were able to resolve bead displacements at molecular dimensions (less than 5 nm), and our approach led to preliminary steps toward development of three unanticipated spin-off technologies that had not been proposed: 3-D bead twisting to measure orthotropic elastic moduli at the single cell level; intracellular stress tomography to image - not structures - but rather to image mechanical stresses transmitted from the bead through the cytoskeleton; and anomalous bead motions to quantify discrete cytoskeletal remodelling events at the nano-scale. In addition, traction microscopy – a technology developed initially by others but advanced by us under separate NIH auspices – now allows us to create high resolution images of mechanical stresses (tractions) transmitted from the cell base to the substratum upon which the cell is adherent. Taken together, these technologies comprise a suite of novel complementary tools that is unequalled in its ability to characterize cytoskeletal mechanics at cellular, subcellular, and molecular levels. These technologies are described in detail below.

To the extent that engineering developments exceeded expectations, biological findings that they enabled did so as well. These technologies led us to a series of unanticipated discoveries with implications for understanding any systems-level biomechanical process stemming from cytoskeletal network dynamics, including embryonic development, remodeling, contraction, wound healing, crawling, metastasis, and invasion. Beyond the specific issue of the airway smooth muscle cell and its role in asthma, basic mechanical questions at the interface of physics, cell biology, and medicine are for the first time amenable to experimental attack.

ISSUES

We are deeply appreciative of the BRP funding mechanism, which enabled us to create new technologies, train young investigators, and carry out bioengineering research that crossed both disciplinary and national boundaries. By their nature, these activities would have been difficult or impossible to support under any other funding mechanism.

We view bioengineering training as a central component of this international consortium, allowing us to develop promising young investigators who are attempting to bridge biology, engineering, and physics. As a measure of our success, we point first to Dr. Ben Fabry, an engineer who was a post-doctoral fellow at Harvard at the beginning of the current BRP grant cycle. On the basis of his accomplishments supported by this BRP, in 2003 he was offered and he accepted a tenured full Professorship at the Institute of Biomedical Technology, University of Erlangen-Nuremberg, Germany. We point to the promising individuals who are currently within our BRP training pipeline or are a product of it:

Dr. Ben Fabry, Professor, University of Erlangen-Nuremberg, Germany.

Dr. Marina Puig de Morales finished her Ph.D. in physics at one consortium partner (Dr. Navajas, U. Barcelona) and in 2001 moved to another (Dr. Fredberg, Harvard U.

Dr. Linhong Deng finished a post-doctoral fellowship with one consortium partner (Dr. Maksym, Dalhousie U), and in 2004 became a Research Associate at another (Dr. Fredberg, Harvard U).

Dr. Xavier Trepat has just completed his Ph.D. in physics (Dr. Farre, U. Barcelona) and is moving in September to a post-doctoral fellowship at another (Dr. Fredberg, Harvard U).

Jack Fairbank and Sarah Connelly are doctoral students supported by this BRP working under the supervision of Dr. Maksym (Dalhousie U.)

PI: Brent A. French, PHD
University of Virginia
Dept. of Biomedical Engineering
415 Lane Rd, MR5 Bldg, Rm. 1219
Health Systems Box 800759
Charlottesville, VA 22903
T: 434-924-5728
F: 434-924-5923
bf4g@virginia.edu

PROJECT TITLE: Gene Therapy for Myocardial Stunning and Infarction

PARTNERS' NAMES AND AFFILIATIONS:
Brent A. French (University of Virginia), Zequan Yang (University of Virginia), Frederick H. Epstein (University of Virginia), Christopher M. Kramer (University of Virginia), Victor E. Laubach (University of Virginia), Klaus Ley (University of Virginia)

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-□, NF-□B, 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-□, inhibiting NF-□B 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-□

knock-outs, in mice with impaired NF-□B 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 successful. 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 therefore reported on these studies at recent meetings of: the American Heart Association, the International Society of Magnetic Resonance in Medicine, and the Society for Cardiovascular Magnetic Resonance.

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
While progress to date has been significant, future advances in cardiac MRI at UVA are largely contingent upon the installation of a contemporary, high-field scanner. All studies to date have been conducted using a ten-year-old 4.7T scanner that lacks the signal-to-noise ratio and gradient rise-times necessary to implement more demanding cardiac protocols (such as perfusion imaging and localized spectroscopy). Members of the Partnership therefore participated in the submission of a proposal to the NCRR High-End Instrumentation Program in September of 2003 for a new 9.4T MR imaging/spectroscopy system. Unfortunately, the application did not receive immediate funding, but was deemed of sufficient merit for further consideration during the next fiscal year.

PI: Charles Gilbert
The Rockefeller University
PI: Charles Gilbert
The Rockefeller University
New York, NY 10021-6399
gilbert@rockefeller.edu

PROJECT TITLE: Imaging Activity in Visual Cortex at the Cellular Level

GRANTING NIH INSTITUTE/CENTER: National Eye Institute

ABSTRACT
Tubby-like proteins (TULPs) comprise a family of proteins found in all multicellular organisms. These molecules are characterized by the presence of a conserved carboxy-terminal "tubby domain" that does not exhibit sequence homology to other known proteins. Genetic mutation of tubby or other TULPs often leads to one or more of three disease phenotypes: (1) obesity - from which the name "tubby" is derived, (2) retinal degeneration, and (3) hearing loss. The disease phenotypes associated with mutations in tubby-like proteins clearly indicate a vital role for these molecules in normal tissue function. While the expression pattern of each family member is distinctive, tubby proteins are found mainly in the nervous system, and all known human tubby proteins are expressed in the retina. Mutation of the TULP1 gene is the cause of retinitis pigmentosa type 14 (about 5 percent of inherited RP cases), and the human TULP2 gene maps within the minimal identified region for the cone-rod retinal dystrophy locus on chromosome 19. Furthermore, tubby mutants bear a remarkable similarity to several human syndromes that result in combined sensorineural hearing loss and retinal degradation, accompanied by obesity. Despite the clear medical importance of tubby-like proteins, no biochemical function has yet been ascribed to any member of this protein family. In order to identify the biochemical function of tubby and other TULPs -and to thus understand their role in disease - we will use X-ray crystallography to determine the high-resolution three-dimensional structure of tubby. We will identify a function for tubby based on structural similarities to other known proteins, by identification of chemical functionalities such as active sites or cofactors, and by the application of concomitant cell biological and biochemical studies, including cellular and sub-cellular localization studies. This is a model problem for a "structural genomics" approach to identification of function for a medically relevant protein. This approach relies on structural information, phenotype data, and classical biology approaches enabled by the availability of pure protein. This type of approach should be greatly facilitated by the recent massive expansion of the database of three-dimensional structures.

STATUS OF RESEARCH AND PARTNERSHIP
We have developed a 2-photon imaging system for long term monitoring of the structure and function of neurons in the visual cortex of the monkey. The system allows imaging many cells at one time, has high spatial resolution for imaging the finest axons, dendrites, spines and axonal boutons, and high temporal resolution for imaging signals related to neuronal activity. The advances in system design include novel optics and scanning technology. Together with the imaging hardware, we have developed fluorescent probes for labeling neuronal structure and for monitoring their function. This involves incorporating genetically encoded probes into viral vectors. In order to use the system for studies of perceptual learning and top-down influences on visual cortical function, we are adapting it for recording from awake, behaving monkeys. We have made progress on several arms of the project, including the refinement of hardware for fast scan multiphoton imaging, developing genetically encoded probes for both structural and activity studies, improvement of viral vectors for delivering the probes, and imaging activity in awake, behaving monkeys.

Fast scan 2-photon imaging. We engineered the scanning heads, lasers and vibration isolation system that can accommodate large animals in a stable framework. Software was added and refined for mapping large areas at high resolution. The system was designed for reproducibly returning to the same sites over multiple imaging sessions. This also required design and implantation of a chamber with inserts that enable us to position the microscope objective to a specific cortical location and that provides a closed environment preventing cortical movement. With this system we have successfully imaged labeled neurons, dendrites and axons in the primary visual cortex of Macaque monkeys. We are testing the activity sensitive probes using the high temporal resolution feature of the microscope. Some of these probes require ratiometric imaging and for imaging dyes that fluoresce at different wavelengths, and we have refined the optics for simultaneous acquisition of signals from different wavelengths.
Genetically encoded fluorescent probes. We have tested new probes and have put genetically encoded probes into viral vectors. These probes will enable us to image both the structural details of neurons and their processes as well as signals related to the activity of neurons.
Developing new viral vectors. We have developed viral vectors, including different viruses and different serotypes of given viruses, in order to optimize the level of expression, tissue and cellular specificity, and duration of labeling
Imaging in awake, behaving macaque monkeys. We have completed one project involving optical imaging in behaving monkeys. This enabled us to visualize, at the population level and at the level of subthreshold activity, interactions between visual stimuli. The technology developed for optical imaging from behaving monkeys can now be adapted to 2-photon imaging.
The system can visualize and create 3-dimensional reconstruction of neurons and their processes over several millimeters of cortex by a "biomapping" technique that sews together numerous z-stacks. The finest processes, boutons and spines can be visualized to a cortical depth of over 400μm. With our registration technique, we can return to the same processes over multiple imaging sessions spanning weeks and months. With our newer viral vectors, the labeling achieved lasts indefinitely, and is therefore useful for studying long term changes in cortical circuits and functional properties. The imaging system has been able to monitor activity as well as structure. We are using it as a test bed to find the best probes for imaging activity in the visual cortex.
In developing the technology for imaging activity in behaving monkeys, we started with the optical imaging technique. The challenge was to be able to maintain the cortex with the dura open for months at a time (all traditional electrophysiological techniques involve recording through an intact dura in order to keep the brain free of infection, swelling and other mechanical and toxic insults). The technical developments have therefore involved surgical techniques, implants and proper maintenance of the environment of the exposed cortical region.
Taken together, these techniques promise to allow us to study long term changes in cortical function and circuitry, the influence of experience and learning, and the interaction between visual experience and top-down cognitive influences. This approach will be applied to studies on the molecular mechanisms of cortical plasticity, and the mechanisms of experience dependent changes, including those of perceptual learning, at the molecular, structural and functional levels.

ISSUES
None

PI: Laurie Gower, Ph.D.
Department of Materials Science & Engineering
University of Florida
Gainesville, FL 32611
T: 352-846-3336
F: 352-846-3355
lgowe@mse.ufl.edu
http://gower.mse.ufl.edu/

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

PARTNERS' NAMES AND AFFILIATIONS:
Saeed Khan, Ph.D. (Pathology, khan@pathology.ufl.edu, T: 352-392-3574), Daniel Talham, Ph.D. (Chemistry), Hassan El-Shall, Ph.D. (Materials Science & Engineering), Richard Dickinson, Ph.D. (Chemical Engineering), Yakov Rabinovich, Ph.D. (ERC for Particle Science & Technology)

GRANTING NIH INSTITUTE/CENTER:
National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK)

ABSTRACT
The objective of the proposed bioengineering research partnership (BRP), located at the University of Florida, is to examine two key issues relevant to urolithiasis; 1) the effects of acidic biopolymers and lipid membranes on nucleation, growth and aggregation of calcium oxalate (CaOx) crystals in an artificial urinary environment; and 2) the injurious effects of a liquid-phase mineral precursor on tubular epithelial cells grown in culture. With regard to 1), many investigators have examined the promotory and inhibitory effects of acidic glycoproteins on crystal growth and aggregation. Our work differs in that a primary focus will be to investigate the relevance of a recently discovered polymer-induced liquid-precursor (PILP) process to pathological biomineralization. The PILP process generates non-equilibrium crystal morphologies which exhibit features similar to crystals found in kidney stones, such as for example, stratified spherulites. Mineral films and coatings are also deposited by the process, and repetitive depositions might lead to concentrically laminated structures, such as those commonly observed in composite stones. In addition, the interfacial aspects of this liquid-liquid phase separation process lead to a pronounced aggregation tendency of crystals. Lastly, we hypothesize that the presence of this cementatious mineral precursor in the urinary tract could influence the attachment and retention of crystals to renal epithelial cells; or the highly ionic precursor phase could cause cell injury or death, leading to the release of modulatory factors or membrane fragments, which could promote heterogeneous nucleation and/or aggregation of crystals. The proposed work consists of 10 Specific Aims which fall under four topical areas: crystal-macromolecule, crystal-crystal, crystal-lipid, and crystal-cell interactions. The bioengineering techniques to be used include measurement of interparticle forces by Atomic Force Microscopy, measurement of long-range interactions between submicron CaOx particles and mimetic lipid membranes with an optical trap force transducer, and nucleation of crystals and PILP phase on mimetic lipid membranes using Langmuir monolayers. This 5-year project will enable us to assess the relevance of the PILP process to pathological calcification, as well as to perform a comparative analysis with the more traditional concepts pertaining to the role of lipids and acidic biopolymers in stone formation, and will contribute to the development of bioengineering techniques that are new to the field of stone research. The long-range clinical goal of this BRP is to provide a more effective means of diagnosis, treatment, and long-term prevention of renal calculi.

STATUS OF RESEARCH AND PARTNERSHIP

Crystal-Macromolecule Interactions: The effects of acidic proteins and mimetic peptides on crystal nucleation and growth have been examined for the calcium phosphate system, with emphasis on the ability to generate the polymer-induced liquid-precursor (PILP) process, which is relevant to both bone formation as well pathological deposits, such as kidney stones, atherosclerotic plaque, and biomaterial encrustations. This year, we have been optimizing the system using the Constant Composition apparatus purchased with this grant, to produce spherical amorphous particles of CaP which are being used in the other studies described below. To establish a firm baseline for crystal nucleation via the traditional crystallization mechanism, statistically designed experiments were used to examine the effect of supersaturation, hyperoxaluria, hypercalciuria, and citrate on nucleation & crystal growth of calcium oxalate monohydrate. Primary nucleation of calcium oxalate monohydrate at different supersaturation ratios was studied. For a better understanding of the primary nucleation and kinetics of growth of COM crystals, the interfacial free energy was determined.

Crystal-Crystal Interactions: Kidney stones frequently contain a calcium phosphate spherulitic core, surrounded by aggregates of calcium oxalate crystals (along with organic matrix). Although spherulites are considered aggregates of crystals, their formation mechanism is entirely different from the aggregation of preformed crystals. The stages of formation of calcium phosphate – calcium oxalate composite aggregates which mimic the microstructure of mixed composition stones were examined using mimetic peptides under conditions simulating the urinary environment.

Crystal-Cell Interactions: Direct Atomic Force Microscope (AFM) measurements of the interaction forces between COM crystals (or silicon nitride tip of the standard AFM cantilever) and a monolayer of renal epithelial cells have been measured. The effect of prior treatment of cells by oxalate solutions was investigated. Adhesion forces have been measured for COM/MDCK interaction, while no adhesion was observed for COM/ LLCPK1-cells, and this difference correlates with the in vivo situation that kidney stones form primarily on MDCK-cells and not on LLCPK1-cells. Cell culture studies have also been used to examine the involvement of reactive oxygen species in calcium phosphate induced renal epithelial cell injury. Our hypothesis, which is supported by studies thus far, is that CaP crystals can independently interact with renal epithelium, promote sites for crystal attachment and then either grow into mature CaP stone or create sites for CaOx crystal nucleation, retention and stone development.

Crystal-Lipid Interactions: Brewster Angle Microscopy (BAM) was used to monitor COM precipitation at phospholipid monolayers that are used as models for lipid membranes. This year's research efforts focused on the role of phase boundaries on heterogeneous COM precipitation at biphasic monolayers. The conclusion of this set of experiments is that COM precipitation only occurs at a phase boundary if there is a dynamic exchange between the molecules in the Liquid Condensed and Liquid Expanded phases. Synchrotron x-ray reflectivity studies of mineral formation under mimetic lipid Langmuir monolayers have been performed at the NSLS. The optical trap force measurement technique has been used to determine equilibrium forces between amorphous micron-sized calcium phosphate PILP particles and a phospholipid (DPPC) bilayer. Based on these experiments, the surfaces coated with a DPPC bilayer appear to enhance the attraction of a calcium phosphate PILP particle and the likelihood for attachment to occur.

ISSUES

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

PI: Robert J. Greenberg
Second Sight Medical Products, Inc.
12744 San Fernando Road, Building #3
Sylmar, CA 91342
T: (818) 833-5000
bob@2-sight.com

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

PARTNERS: University of Southern California (Doheny Eye Institute); Alfred E. Mann Foundation; North Carolina State University; University of California, Los Angeles; Universite de Mons-Hainaut

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

ABSTRACT
Our research for this partnership grant is 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 for use both in research and as a clinical device.

In order 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 basic research that we focus on include:

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

Each of these areas needs additional research for the creation of an optimal chronic retinal prosthesis which 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 make fast progress in its fourth year.
Second Sight has developed three novel electrode materials. Test saline soak data to date can be summarized as:

Second Sight material A – 1013 days at 0.5 mC/cm^2, 468 days at 1.0 mC/cm^2
Second Sight material B – 892 days at 0.45 mC/cm^2
Second Sight material C – 468 days at 2.0 mC/cm^2

In-vivo experiments are planned for the next period.
Chronic stimulation in dogs with a Model 1 (platinum electrodes) with charge densities of up to 0.1mC/cm^2 have shown no retinal cellular damage due to the electrical stimulation for periods of up to 60-120 days of continuous stimulation in sighted and blind dogs. Higher levels of stimulation are under investigation.

More advanced devices with higher electrode counts are still under development.

ISSUES:

After several changes in the partnership, members in the group are working well together. Physical proximity and requiring that subcontractors achieve milestones has led to greater productivity. Making the politically difficult decision to drop a non-performing partner was also very positive for progress.

PI: Henry R. Halperin
Johns Hopkins University
Blalock 524; 600 N Wolfe Street
Baltimore, MD 21287
Office: (410) 955-2412, Fax: (410) 955-0223
Email: hhalper@jhmi.edu

PROJECT TITLE: Magnetic Resonance Guided Electrophysiology Intervention

PARTNERS:
1) Johns Hopkins University [Medicine, Radiology, Biomedical Engineering] (Baltimore, MD)
2) Robin Medical, Incorportated (Balimore, MD)
3) Irvine Biomedical Incorporated (Irvine, CA)
4) ANS Portland (Formerly MicroHelix), Incorporated (Portland, OR)
5) Navicath, Incorporated (Haifa, Israel)

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

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

We hypothesize that MRI, with MRI-compatible (non-magnetic) electrode catheters, catheter-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. We have submitted a competing continuation project that deals 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 (4) Approval of the competing renewal of the project for another 5 years.

We have added an additional partner, NaviCath Incorporated, to develop an MRI-compatible system for remote manipulation of catheters that will allow catheters to be manipulated in patients in MR scanners that are too long to allow easy access to the groin vessels.

Our Investigational Device Exemption covers catheters to be used with low-power MR scans. We have identified a standard MRI-compatible catheter from Irvine Biomedical that can be used with the low power MRI 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.
Other technological developments are continuing. We have obtained improved catheter tip location sensors. These sensors are less than 1.2 mm in diameter. They have been validated in the MR scanner and it has been determined that they provide position accuracy to plus or minus 1 mm. The tip location technology is being incorporated into steerable catheters and the output from the catheter tip location system is being incorporated into a custom scanner control console. The console has already been tested to show that the imaging plane of the scanner can be controlled by the console. The imaging plane will further be controlled by the position information from the catheter tip location system. This technology will allow the imaging plane to be servo-controlled to align itself with the catheter tip, thus to dynamically manipulate the imaging plane to keep it in the plane of the catheter tip.
We have successfully developed 3 dimensional volume and surface rendering software. The data from the catheter tip location system is being interfaced with the 3 D software to allow display of the catheter tip position and direction on the 3 D images of the heart. In addition, we are starting to add simultaneous display of some electrical information to work toward true electro-anatomic maps.
We have completed a study showing that patients with current generation pacemaker and implantable defibrillator devices can undergo MR imaging without complications. This study is important to show that this increasing population of patients (with implantable devices) are still candidates for therapy with MR guided interventions. Previously an implantable device was a contraindication to MRI.
We have demonstrated that MR imaging can give useful information for staging complex ablations. MR imaging pre and post procedure has become the standard of care for atrial fibrillation ablations in our clinical electrophysiology laboratory.
We have studied the use of MRI in identifying patients at risk for ventricular tachycardia (VT), and thereby may be candidates for MR-guided VT ablation. We developed a novel marker of myocardial infarct/scar heterogeneity (the *gray zone*) by contrast enhanced MRI (ceMRI) that is associated with inducible VT at electrophysiologic study (EPS). Patients referred for ICD underwent cine and ceMRI. ceMRI was performed with an inversion recovery fast gradient echo sequence. We defined the *gray zone* as the area of infarct/scar on delayed imaging with signal intensity (SI) > 50% of peak SI within the scar minus the area with SI > 75% of peak SI. All patients had EPS (N=16) per protocol. By the robust Wilcoxon rank-sum, the only MRI predictor of inducibility was gray zone extent ($p=0.005$). Islands of viable myocytes may exist in the periphery of infarct/scar, thus forming the substrate for fatal arrhythmias in ICM. Identifying these regions is possible by differences in SI distribution within the infarct. Areas with less bright SI on delayed imaging likely reflect faster contrast washout because of less dense scar. The gray zone reflects this tissue heterogeneity and appears to be a powerful predictor of inducibility. This type of analysis may lead to identification of patients who are candidates for intervention, including MRI guided intervention.

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

PI: Bruce H. Hasegawa, Ph.D.
Department of Radiology
University of California, San Francisco
UCSF Physics Research Laboratory
185 Berry Street, Suite 350
San Francisco, CA 94107
T: 415-353-9472
F: 415-353-9458
hasegawa@radiology.ucsf.edu.

PROJECT TITLE: Imaging Structure and Function in Small Animals

PARTNERS' NAMES AND AFFILIATIONS: Michael Dae, M.D., (Cardiovascular Research Institute, University of California, San Francisco), Bradley Patt, Ph.D. and Kevin Parnham (Photon Imaging, Incorporated, Northridge, CA), James Carver, (Jamco Engineering, Cottage Grove, OR), Simon Williams, Ph.D. (Genentech, Incorporated, South San Francisco, CA)

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
We are nearing completion of the hardware and instrumentation development phase of the project (Specific Aims 1-3). A major effort, headed by our partner Photon Imaging, Inc., has been devoted to the development of four state-of-the-art 80×80 cadmium zinc telluride (CZT) detectors for radionuclide imaging. 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. Photon Imaging, Inc. is in the process of integrating these first two CZT detectors with read-out electronics and housing for delivery to UCSF in July 2004, where they will be placed in lead enclosures with pinhole collimators for performance tests and initial imaging studies. Photon Imaging then will assemble and integrate the final two CZT cameras for delivery to UCSF by December 2004, and throughout the project will continue to assist UCSF with the design of shielding, housing and collimators, integration of the detector heads into the gantry, and interfacing with read-out electronics.

A second significant effort in this project is devoted to the design and development of a gantry by our partner Jamco Engineering (Cottage Grove, OR). The gantry will include a slip-ring that will rotate a microCT subsystem (x-ray source and CCD camera) and the 4 pinhole SPECT detectors around a horizontal rotational axis. A finite element analysis has been completed to confirm that the proposed design offers sufficient stability and precision for both SPECT and CT. We expect the mechanical accuracy of the system to be maintained within 10 microns with an angular positioning accuracy of approximately 6 arcsec. The rotating stage of the SPECT/CT gantry is scheduled to be delivered to UCSF in early July 2004, on which we will mount both the microCT components (x-ray source, CCD x-ray detector) and the two radionuclide detectors. We will integrate these subsystems with the data acquisition electronics and system control software to test alignment, develop acquisition protocols, and test image quality using phantoms. Jamco Engineering will continue to develop the SPECT/CT gantry components including slip ring, animal handling mechanism, and base unit. These final mechanical units will be developed and integrated with computer interface electronics and system control software to complete the physical elements of the SPECT/CT system by September 2004.

At UCSF, we have developed both analytic (Feldkamp) and iterative (maximum-likelihood expectation-maximization) software to reconstruction tomograms from the microSPECT and microCT data. We currently are developing 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. Our group at UCSF also has assembled and tested the microCT subsystem that uses a low-power microfocus x-ray tube and a high-resolution CCD camera. We have developed software to correct spatial linearities that are introduced by the CCD x-ray detector. Finally, we develop and evaluate 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.

Our BRP includes two specific studies involving small animals that we intend to pursue after we complete the development of the imaging system. In the first, we will perform dual-isotope studies to correlate adrenergic innervation (with ^{123}I-MIBG) and myocardial perfusion (with ^{99m}Tc-MIBI) with transgenic mice that express congestive heart failure: In the second, we will measure tumor and organ distribution of HER2/neu antibody radiolabeled with ^{125}I, in a mouse model that overexpresses HER2/neu. We expect to initiate these studies over the next year of the grant, with the goal of evaluating the SPECT/CT system for murine models of cardiovascular disease, cancer, and other diseases.

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 Photon Imaging) 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.

PI: Ronald B. Hirschl
F3970 Mott Children's Hospital
Ann Arbor, MI 48109-0245
rhirschl@umich.edu

PROJECT TITLE: Total Liquid Ventilation: A Bioengineering Partnership

PARNTERS' NAMES AND AFFILIATIONS:
James Grotberg, Ph.D., M.D.
Biomedical Engineering Department
University of Michigan

GRANTING NIH INSTITUTE/CENTER: NHLBI

ABSTRACT

ARDS is a frequently lethal pulmonary process that occurs in approximately 150,000 patients each year. Total liquid ventilation (TLV), in which the lungs are filled with perfluorocarbon and ventilated with a device which oxygenates and removes carbon dioxide from the perfluorocarbon, has great potential to effectively treat patients with ARDS. The clinical principal investigator has been performing studies in liquid ventilation over the last 8 years. Through our laboratory effort, we have generated data that demonstrate the efficacy of TLV in improving gas exchange, pulmonary function, and oxygen delivery, as well as in reducing acute lung injury. The bioengineering principal investigator has been performing studies in biofluid mechanics and transport of the pulmonary system for many years. This proposal addresses several fundamental physiological and bioengineering issues that underlie the progress toward establishing TLV as a clinical tool: 1) the optimal means for administering the liquid into the lungs; 2) the effect of ventilation parameters upon gas exchange; and 3) the expiratory flow limitation which restricts the effectiveness of the technique. The current research proposal is, therefore, directed at developing a new partnership between a clinician scientist and a bioengineer in the investigation of these issues which involve principles of fluid delivery and distribution, gas transport, and flow limitation during expiration. Specifically, our investigation will assess the distribution of the perfluorocarbon with regard to rate of fill, position during filling, and the characteristics of the perfluorocarbon. Secondly, we intend to investigate and to model the parameters which affect gas exchange during TLV, such as tidal volume, respiratory rate, and lung distension, and to model local flow patterns within the airways and alveoli. Finally, we plan to assess the relationship of flow limitation during expiration to the rate of flow and the state of inflation of the lungs and to investigate strategic means of manipulating parameters which determine flow limitation. A thorough understanding of these issues and solutions to these problems will be critical to the clinical application of this new and exciting technology.

STAUTS OF RESEARCH AND PARTNERSHIP

Our research in the above project has progressed well. First, we have developed data evaluating the effect of rate of perfluorocarbon administration and position with respect to gravity upon the homogeneity of the distribution of perfluorocarbon in the lungs. We have discovered that faster administration of perfluorocarbon and the upright posture enhances the uniformity of distribution. Secondly, we have characterized the effect of perfluorocarbon flow rate and lung volume upon the development of flow limitation during expiration and have defined predictors of the onset of flow limitation. The critical velocity at which flow limitation occurs increases as the lung volume decreases. We are now studying the effects of bronchodilation and alteration of the drainage flow rate as expiration progresses upon flow limitation. We are also developing airway access devices that will minimize airway resistance and are examining the various properties of perfluorocarbons which are associated with the development of flow limitation. Based on the data which we have gathered, we are developing a means for servoregulation of

liquid drainage from the lungs and avoidance of airway collapse. Third, our group has developed models of gas exchange in the alveolus partially filled with perfluorocarbon. We have assessed the relationship of ventilatory parameters in-vivo during TLV to gas exchange and have used these data to model alveolar gas exchange in the totally perfluorcarbon-filled lung. Such models reveal that convection plays a significant role in gas transport in TLV, and predict that ventilation parameters, such as I:E ratio, frequency, tidal volume, and inspiratory dwell time can modify the resulting flow and gas exchange. We will be applying these parameter predictions to studies involving lung-injured animals in order to assess the effect upon gas exchange efficiency during TLV. Finally, we have developed and successfully tested a variety of devices to perform TLV including those which for the first time employ solid silicone hollow fiber gas exchange devices. We are now defining the parameters of the gas exchange device which determine optimal gas exchange efficiency during TLV.

ISSUES

We hold conferences involving all members of the partnership, along with trainees, every 2 to 4 weeks. Ongoing research is discussed and data presented at these meetings with vital input contributed from both the bioengineering and clinical investigators. These conferences, with the associated integration of expertise, have resulted in a broader approach to our research.

We have numerous trainees involved in the partnership including two M.D. research fellows and two bioengineering post-doctoral students. A bioengineering junior faculty spends a significant portion of his time in the animal laboratories of the clinical partner. Together, all of these trainees have been critical to sustaining the cross-fertilization and integration that has been valuable to this partnership.

We have no specific problems with the partnership.

PI: Eric A. Hoffman, Ph.D.
Professor of Radiology and Biomedical Engineering
Department of Radiology
University of Iowa Carver College of Medicine
200 Hawkins Drive
Iowa City, Iowa 52242
eric-hoffman@uiowa.edu

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; Secondary: National Cancer Institute.

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."
- 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.

PI: Raymond Houck
CEO
Automated Cell, Inc.
390 William Pitt Way
Pittsburgh, PA 15238
T: 412-826-5250
F: 412-826-5215
rhouck@automatedcell.com
www.automatedcell.com

PROJECT TITLE: Quantitation of Cellular Protein Production in Real Time

PARTNERS' NAMES AND AFFILIATIONS: Tao Cheng, M.D. (University of Pittsburgh Cancer Institute), Julie Goff, M.S. (University of Pittsburgh Cancer Institute), Julie Glowacki, Ph.D. (Harvard Medical School), William Gooding, M.S. (University of Pittsburgh Cancer Institute).

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

ABSTRACT
This partnership focuses on development of automated technologies for gathering and interpreting information about marker and secreted protein expression at the individual cell level. Our biomedical research partnership combines the technological expertise of Automated Cell, Inc. (ACI) with the biomedical expertise of researchers at University of Pittsburgh Cancer Institute (UPCI) and Harvard Medical School (HMS) to focus on the development of tools for real time study of individual cells and mixed populations of cells in an automated combinatorial cell culture system.
Goals include: 1) Development of software for real time analysis and dynamic manipulation of osteoblast progenitor/precursor cells; 2) Development of devices and procedures for coherent real time detection of phenotypic changes in rare human cells and the progeny of such cells deposited, tracked, imaged and analyzed individually through time; 3) Innovation of technologies for real time quantitation of cellular protein production through miniaturized assay methods and off-line analytical systems; and 4) Application of these technologies to determine effects of aging on osteoblast differentiation potential of human bone marrow stromal cells, utilizing the STRO-1+ subset, in an in vitro model system.

STATUS OF RESEARCH AND PARTNERSHIP:
In the second year of this partnership we have made significant progress on all four specific aims and have continued to work closely to combine technology development in automated live cell analysis with ongoing investigation of osteoblast cellular biology. Software and hardware innovation has evolved particularly around goals involving fluidics-based protein micro-assay technologies and assembly of a new automated system at the ACI facilities that incorporates features specific for this project. Application of image and data analysis software improvements from last year has led to a completed study of motility and proliferation of osteoblast-type cells (Bahnson et al, submitted). Improved methods for isolation and culture of purified STRO-1+ cells from osteoporosis patient bone marrow are being investigated at the UPCI facilities using both traditional and automated image-based approaches to analysis.
We have constructed and demonstrated effectiveness of multi-port needles that provide improved efficiency for fluid handling in 384 well plates. Software development in this area includes interfacing and coordinating controls for multiple syringes that simultaneously add and remove stain, wash, and culture medium replacement solutions. These proprietary techniques and methods provide advantages over single-port needles in terms of operation time and solution replacement efficiency for serving multiple wells with minimal disruption of cells. Simulated double-antibody staining has demonstrated the potential for procedures with complex sequences to be applied through these methods. In related

investigations we have demonstrated successful multi-well dispensing of micro-beads by automated fluidics and have performed reliable recovery of selected individual beads using software controls that may be adaptable to automation in the future. These advances go a long way toward realization of technological goals in our original application and we anticipate groundbreaking studies over the coming year as we implement them in a repeatable controlled fashion for study of osteoblast differentiation in the automated culture systems at ACI and UPCI.

The newly constructed system at ACI incorporates features developed on the fluidics workstation that will now be implemented in the controlled environment with cells being monitored in continuous culture. This system is adaptable for both in-line fluidics manipulation using the multi-port single needle and for transfer of the plate to off-line devices, depending upon demands of the experiment. Under design is an off-line device incorporating multiple needles for more rapid servicing of wells in 384 well plates. Flexibility for automated plate transfer also broadens possibilities for studies involving multiple plates, a feature which may be particularly advantageous for single-cell studies with low seeding efficiency. The new system at ACI and a system upgrade at UPCI will also utilize fluorescence excitation sources that provide more uniform illumination for quantitative fluorescence analysis.

Bone marrow samples from hip-replacement patients with osteoporosis are being obtained from Harvard Medical School and shipped to UPCI for enrichment of STRO-1+ cells and plating under various conditions in the combinatorial cell culture system. Low recovery from initial experiments using FACS purification has prompted the team to seek improvements using paramagnetic bead technologies for pre-enrichment, with indications that this approach yields higher numbers of osteoblast differentiated alkaline phosphatase positive STRO-1+ cells. Image sets are currently being analyzed for quantitative phenotype indicators, and baseline staining intensities are being established using manual methods for comparison to automated fluidics-mediated methods to be brought on line at UPCI after successful implementation at ACI.

ISSUES

Software development will continue to focus on remaining steps for updating experimental outcomes in real time in order to link outcomes to dynamic initiation of automated events, for example staining of wells that show activated cell migration, or staining and addition of growth factors timed upon cell division. Analytical improvements such as pixel-based quantitation of motion ("optical flow"), persistence of migration, bead fluorescence intensity and cell proximity analysis will be incorporated as development along these lines progresses. Progress in automation of quantitative analysis of fluorescence intensity for both cell phenotyping and bead-based analysis for secreted proteins is expected to improve substantially with more stable fluorescence illumination in the new system.

The dedicated system now coming on line will fully integrate fluidics functionality and quantitative fluorescence analysis of living cells in the controlled culture environment. Completion of this system was delayed by budget alterations initiated at the time of grant approval.

We will routinely implement methods developed on the fluidics workstation into long-term experiments; these pioneering studies will demonstrate the nature of time dependent changes in surface marker expression and effects of repeated staining at the individual cell level. At UPCI, STRO-1+ cell purification and culture methods will continue to be optimized, and a fluidics unit will be assembled for this system incorporating hardware and software demonstrated to be effective at ACI.

PI: David Huang, M.D., Ph.D.
University of Southern California
Doheny Eye Institute
1450 San Pablo Street, Box 5705A
Los Angeles, CA 90033
(323) 442-6710
davidhuang@alum.mit.edu

PROJECT TITLE: Advanced Imaging for Glaucoma

PARTNERS' NAMES AND AFFILIATIONS:
David S. Greenfield, MD (Bascom Palmer Eye Inst., Univ. of Miami, Miami, FL)
Joseph A. Izatt, PhD (Dept. of Biomedical Engineering, Duke Univ., Durham, NC)
Robert W. Knighton, MD (Bascom Palmer Eye Inst., Univ. of Miami, Miami, FL)
Andrew M. Rollins, PhD (Dept. of Biomedical Engineering, Case Univ., Cleveland, OH)
Joel Schuman, MD (University of Pittsburgh Medical Center, Pittsburgh, PA)
Scott D. Smith, MD, MPH (Cole Eye Institute, Cleveland Clinic, Cleveland, OH)

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. 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 1 of 5. Dr. Huang's team has developed new 3-dimensional grid scanning patterns and image processing algorithms for imaging the optic nerve head and macula with the current high speed commercial OCT system (Stratus OCT, Carl Zeiss Meditec, Inc., 400 axial scans per second). The software improves the measurement of the neural tissue in the optic nerve head and the inner retinal layers in the macula, both of which are anatomic indicators of glaucoma. These new methods are being tested in a longitudinal clinical trial that compare OCT with other advanced imaging methods (scanning laser polarimetry and tomography) and standard glaucoma diagnosis with visual field testing and optic nerve head photography.

Dr. Izatt's team has developed an ultrahigh speed (10,000 axial scans per second) and resolution (6 microns) Fourier domain (FD) OCT system and demonstrated retinal imaging *in vivo*. This technology will be ported to a clinical retinal scanner for the clinical study in Year 2. It is anticipated that the improvement in speed and resolution will greatly enhance anatomic measurements.

Dr. Rollin's team has developed an OCT system that scans both transverse position and incidence angle. This system is being tested with phantom targets and *ex vivo* retina preparations to characterize the directional reflectance of the retinal nerve fiber layer.

Dr. Knighton's team has further advanced methods for characterization of rat *ex vivo* retina preparations with thickness, directional reflectance and birefringence measurements using scanning laser microscope, microreflectometry and micropolarimetry. These measurements will be used to validate Dr. Rollin's angle-resolved OCT system and Dr. Izatt's ultrahigh resolution and birefringence OCT system.

ISSUES

The initiation of patient recruitment in the clinical study was delayed due to the time needed to install advanced imaging instruments and software at all Clinical Centers, to write a detailed Manual of Procedures and to train and certify study personnel. These activities are now complete and recruitment is being ramped up for completion during Year 2 as planned. The grant is being transferred from the Cleveland Clinic to University of Southern California (USC) at the start of Year 2. This presented a budgetary challenge because of the additional personnel needed to establish a Clinical Center at USC and because USC has a higher F&A rate. A request was submitted to allow an increase in total cost while maintaining a slightly reduced direct cost. The additional USC Clinical Center will help recruitment of the planned 800 study subjects and inclusion of Hispanics.

The study initially did not include ultrahigh speed OCT with FD technology in its specific aims. However, the investigators feel that this technology is very important for advancing glaucoma diagnosis and management. The addition of this new goal slightly delayed the introduction of advanced OCT hardware into the clinical study. But the added improvement in sampling density and reduction in motion error is well worth the delay.

PI: Jay D. Humphrey
Department of Biomedical Engineering
233 Zachry Engineering Center, 3120 TAMU
Texas A&M University, College Station, TX 77843-3120
T: 979-845-5558
F: 979-845-4450
jhumphrey@tamu.edu

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

PARTNERS' NAMES AND AFFILIATIONS:
Texas A&M Dwight Look College of Engineering:
J.D. Humphrey, Ph.D., K.R. Rajagopal, Ph.D.
Texas A&M College of Veterinary Medicine:
T.F. Fossum, D.V.M., Ph.D., M. Miller, D.V.M., J. Stallone, Ph.D.
Texas A&M Health Science Center:
G. Davis, M.D., Ph.D., L. Kuo, Ph.D., E. Wilson, Ph.D.
Washington University, Department of Biomedical Engineering:
L.A. Taber, Ph.D.

GRANTING INSTITUTE: National Heart, Lung, and Blood Institute (HL-64372)

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

exploration of further hypotheses in an efficient manner and guide pharmacologic delivery strategies. Years 1-2 will focus on the time-course of changes due to the development of hypertension whereas years 3-5 will focus on the time-course of changes due to reversing the hypertension either mechanically or via specific pharmacological agents, both as a function of the time during the development of the hypertension and the time that the intervention is initiated.

STATUS OF RESEARCH AND PARTNERSHIP

There have been no changes in the partners or the organizational structure. As noted by the study section, the greatest promise and yet the greatest challenge in this research project is the attempt to synthesize data from such diverse sources (clinical, biomechanical, physiologic, cell biologic, histochemical, and molecular) into a mathematical model having both descriptive and predictive capability. Consequently, the first need was development of a general framework for the model based on observed changes in vascular structure and function due to hypertension. This development continues, but we have made significant progress as documented in five papers. Briefly, we have shown that one can exploit advantages of a full nonlinear continuum mixture theory that accounts for changing mass fractions, natural configurations, properties, and rates of turnover of the predominant structural constituents while avoiding potential complications thereof that are associated with the need to prescribe boundary conditions on (mathematical) partial tractions, etc. This is accomplished via a homogenization of the stress response function that allows one to treat the vascular wall as a constrained mixture and thus ignore possible momentum exchanges between constituents. The constitutive structure further reveals those quantities (e.g., rates of constituent turnover) that need to be measured as a function of radial location in the wall and time of development. We also needed to develop new monoclonal and polyclonal antibodies to test the hypothesis that some of the hypertensive changes in the arterial wall mimic changes seen in wound healing responses. These antibodies have now been developed and early data suggest that our hypothesis is correct. We have nearly completed the proposed study of the time-course of changes at 2,4,6 and 8 weeks of hypertension and are now beginning to study the time-course of changes due to mechanical reversal of the hypertension. Albeit not anticipated, interesting cell biologic changes in the vicinity of the coarctation, both in the endothelium within the distal disturbed flow region as well as in the adventitial collateral vessels, is providing additional information on complex growth and remodeling responses.

ISSUES

The primary “issue” in this BPR is that the project is structured such that 5 different labs must simultaneously collect tissue and perform tests on 7 different vessels from all of the animals, which are maintained from 3 to 10 weeks post-surgery. Clearly, not every lab gets “good” data from every experiment, hence it has been a challenge to optimize surgery and necropsy schedules so as to maximize the use of the micro-pigs in providing sufficient data for each lab while not unnecessarily duplicating particular study groups. Overall, however, the cost-and scientific-benefits of having tissue from multiple sites in the same animals outweighs this.

PI: Prof. Marcos Intaglietta
Department of Bioengineering
University of California, San Diego
9500 Gilman Dr.
La Jolla, CA 92093-0412
T: (858) 534-4275
F: (858) 534-5722
mintagli@ucsd.edu

PROJECT TITLE: Bioengineering design of artificial blood

PARTNERS:
Prof. Vladimir Torchilin
Department of Pharmaceutical Sciences
Northeastern University, Boston, MA

Dr. Robert M. Winslow
President
Sangart Inc., La Jolla, CA

Prof. Marcos Intaglietta (P.I.)
Department of Bioengineering
University of California, San Diego
La Jolla, CA 92093-0412

GRANTING INSTITUTE: Heart, Lung and Blood Institute

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. Different OCPEs will have different effects in this process leading to different types of vasoactivity. The product is now finalized and was tested by Sangart, Inc., San Diego, in clinical trials phase 1 at the Karolinska Institute of Stockholm. The trial was successful and phase 2 trials are being designed.

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. For the period of 2 hours following resuscitation, Mal-PEG-Hb was 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 clinical trials that were successful. The material is now in Phase 2 clinical trials in Sweden, and has been approved for clinical trials in the US.

ISSUES
No issues

PI: Steven Jacques, PH.D.
Oregon Health & Science University
Biomedical Engineering/Dermatology
20000 NW Walker Rd., Beaverton, OR 97006
T: 503-748-1512
F: 503-748-1406
sjacques@bme.ogi.edu
http://www.bme.ogi.edu/biomedicaloptics/

PROJECT TITLE: Biomedical Optics for Medical Research and Clinical Care

PARTNERS' NAMES AND AFFILIATIONS:
Sean Kirkpatrick (OHSU Biomedical Engineering), Ken Lee MD, Molly Kulesz-Martin (OHSU Dermatology), Fred Nuttal (OHSU Hearing Center), William Horton MD (OHSU/Shriners Childrens Hospital), Blair Jobe (OHSU Surgery), Scott Gustafson DVM (Oregon State Univ., Veterinary Medicine), James McNames (Portland State Univ.), Scott Prahl (Oregon Medical Laser Center/Providence St. Vincent Hospital), Tim Thomas (TKD, Inc.), Buzz Hill (Minds I Inc.)

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

ABSTRACT
The goal is to establish a consortium of investigators on the medical school campus, Oregon Health & Science University (OHSU), and engineering center campuses in Oregon. Aim 1: Establish a Biomedical Optics Laboratory on the OHSU campus as a central resource supporting the interface of engineering centers with biomedical research and clinical studies. Aim 2: Initiate biomedical optics projects in the field of tissue engineering and biomaterials development. Aim 3: Initiate biomedical optics projects in the field of cancer detection, imaging and treatment, and cell biology. Aim 4: Develop the partnership between bioengineering and medical research by establishing a program identity that reinforces formal communication amongst participating investigators, staff, and students and represents a visible program to potential new collaborators in the medical centers.

STATUS OF RESEARCH AND PARTNERSHIP
Aim 1: The Biomedical Optics Laboratory is now established and actively engaged in projects throughout the medical school, catalyzing new collaborations and fostering an awareness of the potentials of biomedical optics throughout the medical school community. The laboratory is established with space and resources within a cell and molecular biology laboratory in the Dept. of Dermatology. Engineering center collaborators include the Oregon Medical Laser Center at Providence St.Vincent Hospital and the Oregon Graduate Institute (Dept. of Biomedical Engineering) that merged with OHSU to form a new School of Science and Engineering.

Aim 2: We have pursued several biomedical optics projects:
2.1. Novel combined fluorescence-mode and reflectance-mode confocal microscopy system, with a novel pinhole configuration to enable deeper imaging despite tissue scattering.
2.2 Polarized light imaging of skin cancer to guide Mohs surgery.
2.3 Optical fiber spectroscopy of skin and other tissues, introducing novel catheter designs and basic theory on how fiber collection efficiency is affected by tissue optical properties.
2.4 Photodynamic therapy of cutaneous sarcoids in horses, and other veterinary applications including the sterilization of wounds infected with drug-resistant bacteria.
2.5 Optoacoustic imaging of vascular and melanotic skin lesions.
2.6 Optical coherence tomography and its relationship to tissue optical properties.

2.7 Low-coherence interferometry to monitor vibrations of the cochlear membrane of the inner ear.
2.8 Optical measurements of strain using laser speckle in engineered tissues and applied to cells with optical tweezers exerting the driving force.
2.9 Dynamic calibration of pulsed oximetry (pOx) to enable accurate pOx even at low oxygen levels.
2.10 Optical spectroscopic monitoring of stomach-esophageal anastamoses to follow the vascularization required for viability and the fibrosis required for strength.

Aim 3: Partnership development. We have established collaborative relationships with local small businesses (TKD Inc. and Minds I Inc.) to assist in the translation of projects 2.2, 2.4, and 2.9 into robust systems for clinical use. We have developed a collaborative research project (2.9) with an undergraduate school, Portland State University (Dept. of Electrical Engineering). We have held an annual local Biomedical Optics Symposium as part of the Oregon Academy of Sciences meeting.

ISSUES

Our BRP sought to meet the concept of a "partnership" between the medical school community (both medical research and clinical care) and the engineering community (various engineering schools, research centers, small businesses). The goal was as much to establish a community network with an identity that attracted an increasing number of collaborative projects, as to pursue particular scientific projects. We knew the key was to foster communication between two distinct communities (medical versus engineering). This strategy has been successful. We have established the identity of the Biomedical Optics Laboratory as a resource center within the university and continue to recruit new investigators and clinicians into our partnership. This effort, we believe, has met the spirit of the Bioengineering Research Partnership program, yielding an active and enduring community, even as the partnership has advanced a set of scientific projects.

PI: Rakesh K. Jain
Massachusetts General Hospital and Harvard Medical School
Edwin L. Steele Laboratory, Department of Radiation Oncology
Massachusetts General Hospital
100 Blossom Street, COX 7
Boston, MA 02114
T: 617.726.4083
F: 617.724.1819
jain@steele.mgh.harvard.edu

PROJECT TITLE: Integrative Biology of Tumor Angiogenesis, Invasion and Metastasis

PARTNERS:
Project 1: Vascular Angiogenesis
Dai Fukumura, MGH **Donald G. Buerk**, University of Pennsylvania **Paul L. Huang**, MGH/Harvard **Peter T.C. So**, MIT **Raju Kucherlapati**, MGH/Harvard
Project 2: Invasion
Yves Boucher, MGH **Michael Klagsbrun**, Children's Hospital **Bruce Zetter**, Children's Hospital **Jack Lawler**, Beth Israel Deaconess Hospital
Project 3: Hematogenous Metastasis
Lance L. Munn, MGH **Josh Fidler**, MD Anderson **Brian Seed**, MGH/Harvard
Project 4: Lymphangiogenesis and Lymphatic Metastasis
Rakesh K. Jain, MGH **Kari Alitalo**, Helsinki, Finland **Peter Carmeliet**, Leuven, Belgium **Peter T.C. So**, MIT **Noah Choi**, MGH/Harvard **Eugene Mark**, MGH/Harvard **Douglas Mathisen**, MGH/Harvard **John Wain**, MGH/Harvard **Errki Ruoslahti**, Burnham Institute, LaJolla, CA **David Jackson**, Oxford, UK **Stanislav Tomarev**, NIH **Annick Van den Abbeele**, Dana Farber Cancer Institute **George Demetri**, Dana Farber Cancer Institute **Raju Kucherlapati**, MGH/Harvard

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

ABSTRACT
Now that numerous important genes associated with tumor angiogenesis, invasion and metastasis have been discovered, the grand challenge is to understand their function in intact animals. The second major challenge is to integrate and apply this knowledge to cancer prevention, detection and treatment. In the proposed BRPG, we will meet these challenges with a new, more precise, quantitative, integrative and multi-disciplinary bioengineering approach. This new bioengineering approach builds on unique and innovative techniques such as 1) genetically engineered mice to visualize gene expression, 2) in vivo models to visualize molecular and cellular events, 3) computer-assisted in vivo microscopy to quantify gene expression and function continuously and non-invasively at high (1-10 μm) resolution in intact animals, 4) mathematical modeling to integrate the resulting information. Using this powerful technology, we will investigate four critical aspects of tumor metastasis: angiogenesis, invasion, hematogenous metastasis, and lymphangiogenesis & lymphatic metastasis. In the first year we achieved several significant milestones: i) critically tested the long-standing but unproven hypothesis that angiogenesis facilitates metastasis by increasing cell shedding; ii) established a quantitative link between cell traction force and invasion through the tissue matrix; iii) lymphatics are not present inside human tumors; iv) provided the quantitative relationship between nitric oxide (NO) and angiogenesis in vivo. In the second and third years we built on these findings to provide deeper quantitative insight into expression and function of three genes (NO synthase, VEGF-A, VEGF-C) considered critical to these four aspects of metastasis. We also showed that lymphatic metastasis occurs from functional lymphatics in the tumor margin. In the fourth year, we began to collect evidence that host-tumor interaction modulate metastatic processes, successfully showed the effect solid stress on vascular compression as well as gene expression profile change, and established new animal models for lymphangiogenesis and metastasis. Year five will see integration of these data in a unified framework and identification of strategies for clinical translation. The proposed BRPG offers a new paradigm for integrative studies of the dynamics of gene expression and function in cancer. With

this new paradigm available to our collaborating partners working at the forefront of genomics and proteomics, this BRPG will facilitate translation of knowledge about the molecular biology of cancer into effective cancer prevention, detection and treatment strategies.

STATUS OF RESEARCH AND PARTNERSHIP (July 1, 2000 – present)
Project 1: Vascular Angiogenesis: In collaboration with Dr. Donald G. Buerk, we found that nitric oxide (NO) mediates angiogenesis in solid tumors and highly metastatic variant tumors produce more NO and exhibit more but smaller tumor vessels. Chronic NO inhibition resulted in larger and less tortuous vessels. In collaboration with Dr. Paul L. Huang, we discovered that eNOS but not iNOS in host stromal cells contributes to angiogenesis and vessel morphogenesis in tumors. We also uncovered paracrine regulation of angiogenesis and adipogenesis through VEGFR2 signaling paving the way for understanding the link between obesity and cancer. Using transgenic mice expressing GFP under the control of the various promoters, we found that implanted host cells survive, proliferate and contribute to tumor angiogenesis and growth suggesting host stromal cells can provide provisional stroma for metastasizing tumor cells during their initial survival, angiogenesis and growth.
Project 2: Invasion: By using cellular and molecular reagents developed by Drs. Michael Klagsbrun and Bruce Zetter, we have shown that VEGF plays a dose-dependant role in cancer cell mobility. The degradation of collagen type I telopeptides (non-helical domain that participates in collagen polymerization) enhances tumor cell invasion. The enhanced invasion was related to a reduction in collagen rigidity. Using second harmonic generation microscopy and GFP expressing tumor cells, we showed that amoeboid protrusion and invasion are enhanced in collagen I gels polymerized without telopeptides. In stiffer, telopeptide-intact collagen gels, tumor cell deformability is significantly restricted. These results imply that the structural organization and stiffness of fibrillar collagen I can regulate the amoeboid motion of tumor cells.
Project 3: Hematogenous Metastasis: By using cellular and molecular reagents developed by Drs. Josh Fidler and Brian Seed, we have established an orthotopic model of renal cell carcinoma in mice that allows us to measure the rate of cell shedding by a renal tumor. The transcriptional and functional analyses revealed that CD44, □3 integrin and caveolin were downregulated in the shed tumor cells and shedding is a passive process facilitated by reduction in cell attachment through □3 integrin or CD44. By using our compression device, we have conducted a survey of cancer cells and glioblastomas and looked at their differential gene expression in response to solid stress. In response to 24 hours of compression, we have detected changes in nine genes related to tumor suppression, migration, angiogenesis and adhesion. We have also investigated the role of the interstitial matrix in the growth of tumor spheroids and related this to their metastatic potential. Our results suggest that the gel elicits a biological response that increases the adhesion between cells, which is mediated by the interstitial matrix produced by the cancer cells.
Project 4: Lymphangiogenesis and Lymphatic Metastasis: In collaboration with Dr. Kari Alitalo, we discovered that intratumor lymphatics do not function despite the presence of the lymphangiogenic molecule VEGF-C and its receptors VEGFR2 and R3 in tumors. Furthermore, in collaboration with Dr. Peter Carmeliet, we showed that VEGF-C increases angiogenesis and growth in tumors without altering leukocyte-endothelial interactions. In collaboration with Drs. David Jackson and Dr. Stanislav Tomarev, we showed that the recently discovered lymphatic marker (LYVE-1) is also present in the sinusoidal blood vessels of the liver, but absent in the primary and secondary liver tumors. We then used two-photon microscopy to image deeper functional lymphatics and found them to be absent in both animal and human tumors. We also demonstrated that these tumors still metastasize to lymph nodes, in spite of the lack of functional intratumoral lymphatics, suggesting that lymphatics in the tumor margin are the pathway for lymphatic metastasis. This paradigm was confirmed in human lung cancers, which lacked intratumor lymphatic vessels, yet still had lymph node metastases. We also showed that VEGF-C overexpression did not induce the formation of functional intratumor lymphatic vessels, but did cause lymphatic abnormalities that altered proper lymphatic flow. Additionally, we have identified nitric oxide as regulator of the function of the lymphatic system. Nitric oxide controls the tone and contraction of collecting lymphatic, leading to a decrease in lymph flow when the production of nitric oxide is inhibited. Finally, we have shown that compressive mechanical forces generated by proliferating tumor cells can collapse both blood and lymphatic vessels and render them non-functional.

ISSUES: None.

PI: John Johnson, Ph.D.
The Scripps Research Institute
Department of Molecular Biology
10550 North Torrey Pines Road
La Jolla, CA 92037
T: 858-784-9705
F: 858-784-2980
jackj@scripps.edu
http://noda.scripps.edu/

PROJECT TITLE: Plant viruses as platforms for biomaterials

PARTNERS' NAMES AND AFFILIATIONS:
M. Young (Montana State University, Bozeman, MT), T. Lin (The Scripps Research Institute, La Jolla, CA), A. Zlotnick (University Oklahoma Health Sciences, Oklahoma City, OK), M.G. Finn (The Scripps Research Institute, La Jolla, CA), P. Doerschuk (Purdue University, West Lafayette, IN), T. Douglas (Montana State University, Bozeman, MT)

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
Work has been initiated between members of this partnership and significant progress has been made towards achieving the goals of the project. In particular the following interactions have been initiated: Johnson-Doerschuk image reconstruction; Johnson-Finn development of CPMV constructs and attachment of organics to CPMV; Young-Douglas development of CCMV constructs and attachment of organic and inorganics to CCMV; Johnson-Young-Douglas image reconstruction of CCMV constructs; Johnson-Young-Zlotnick development of CCMV assembly system.
Members of the partnership have participated in two focused meetings as opportunities for presenting current and future research and a third is planned for Summer 2004. One of these meetings was held in Big Sky Montana (September 2002) and included members of the partnership (P.I.s) and selected individuals with a significant interest in the use of protein cages as templates for nano-materials (NASA-Ames, UC Berkeley, The Scripps Research Institute, Montana State University). A total of 25 investigators were in attendance. The second meeting was held in LaJolla at The Scripps Research Institute and included all P.I.s from the partnership, post-docs, graduate students, and undergraduates. Additional investigators, outside the partnership, with an interest in the broad goals of the research were

invited and encouraged to attend (Rice Univ, UCLA, Caltech, Montana State, The Scripps Research Institute). There were 85 people in attendance at this meeting. The planned Summer 2004 meeting will occur as the Conference on Viruses and Protein Cages as Materials at Montana State University during August 1-3, 2004. As in the past, interested investigators outside of the grant have been invited and encouraged to attend, including post-docs and graduate students, and about 100 people are expected.

ISSUES

As evidenced by the activities of researchers beyond the partnership, and their interest in participating in the meetings the partnership has organized, it is clear that there is a growing interest in the use of viral capsids (and related protein cage structures) as templates for a new generation of nano-materials. How can the partnership respond to this interest, through additional collaborations/interactions without losing the focus and synergy among members, which is a very strong aspect of this partnership?

PI: Andrew Karellas,, Ph.D.
Professor of Radiology
Emory University
Winship Cancer Institute, Radiology
Building C, Suite 5018
1701 Uppergate Drive
Atlanta, GA 30322
E-mail: akarell@emory.edu
Tel: 404-712-2411 Fax: 404-712-5813

PROJECT TITLE: DIGITAL MAMMOGRAPHY WITH A HIGH RESOLUTION FLAT PANEL IMAGER

PARTNERS' NAME AND AFFILIATIONS: Fairchild Imaging (formerly, Lockheed Martin Fairchild Systems) 1801 McCarthy Blvd, Milpitas, CA 95035.

GRANTING INSTITUTE: NIBIB, formerly NCI

ABSTRACT
At the present time, there is no consensus on the spatial resolution requirements for an optimized digital mammography system and this has been one of the motivating factors for this research. There are currently no large-area (non-scanning), high-resolution (below 70-micron pixel size) digital imaging systems with variable resolution capability for mammography. Our prior experience (not part of this research) with the 100-micron pixel, GE clinical evaluation prototype in a screening population suggests equivalence for cancer detection with similar sensitivities. However, there are concerns about the ability to detect more subtle forms of microcalcifications such as subtle and amorphous. In studies where film and digital is available and direct comparison can be made, these subtle calcifications can be very challenging to visualize with digital mammography. When calcifications are visualized, their edges do not appear to be as sharp as that observed with spot film views, and this may be attributed to the relatively large pixel size of conventional digital imaging sensors.
This bioengineering research partnership with Fairchild Imaging (formerly, Lockheed Martin Fairchild Systems) is aimed at developing and evaluating a new high-resolution flat-panel mammographic imager with variable pixel size (40 and 80 microns). We have developed and tested a monolithic CCD imaging module 8x8-cm with a fundamental pixel size of 39-microns. This device does not have variable resolution capability at this time. This next generation imager, that is currently in progress, is a 2 x 3 array of large-area CCDs tiled in a seamless fashion to provide an imaging area of 16 cm x 24 cm with resolution capabilities of 39 and 78-microns. The CCD array is coupled to a structured CsI:Tl scintillator using non-tapering (1:1), straight fiberoptics, thereby preserving the spatial resolution without the detrimental loss in the collected signal, which is common with the older generation that use tapered fiberoptics. Hence, this investigation was undertaken with the specific hypotheses stated in the application. The research plan calls for preliminary computational and experimental studies followed by development and comprehensive evaluation of the system through objective and universally accepted metrics, such as the modulation transfer function (MTF) and detective quantum efficiency (DQE). In addition, we will explore perceptual metrics such as contrast-detail (CD) characteristics of the system and compare it to existing clinical mammography systems.

STATUS OF RESARCH AND PARTNERSHIP:

The program began on July 1, 2001 and the following research activities have been accomplished:

1. Model: A mathematical analysis approach is currently being investigated to theoretically predict the potential imaging characteristics of the proposed system that can then be used to study the device under various conditions. This mathematical model is a variation of a method that has been used previously by other investigators in the field. Preliminary results indicate good correlation between the mathematical simulation and experimental results with the single module CCD device. The mathematical analysis is not the main focus of the research, but it can be very instructive in the understanding of the effect of certain parameters on the performance of the system, and helps refine and redesign certain elements of the imaging detector to enhance its performance.
2. Development of a single module prototype: A single module 8x8-cm prototype, which operates at a pixel pitch of 39-microns, has been developed and is currently being investigated under clinically relevant x-ray spectral conditions. Two prototypes were developed for investigational purposes. In the first prototype we faced electronic noise issues that were rectified in the second version. This 8x8-cm monolithic module is currently being studied to ascertain the final specifications for the large-area imager. Specifically, we are focusing on MTF, DQE, electronic noise and system stability.
3. Scintillator/system evaluation: Structured CsI:Tl scintillators from two vendors varying in thickness and coating type were evaluated. We have now arrived at a preferable scintillator thickness of 150-160 microns as a compromise between resolution and detective quantum efficiency (DQE). By using our prototype that consists of a single module 8 x 8-cm CCD, we attained an MTF of about 10 cycles/mm at the 10% level and a DQE of close to 55% at 4.7, 9.5 and 19.7 mR of exposure on the detector (clinical range of exposures in mammography). These results are in agreement with our expectations. We were able to attain a DQE that is comparable to other FDA approved digital mammography systems with a much greater spatial resolution.
4. Fiberoptic faceplate evaluation: The appropriate thickness of fiberoptic plate required to provide adequate protection to the CCD has been identified and incorporated into the single module prototype, and we have published a manuscript based on this work.
5. Large Area imager: Our partner, Fairchild Imaging, is in the final stage of manufacturing a large area 16 x 24 cm imager. We will perform comprehensive physical characterization and image quality evaluation with this device. The individual modules will be evaluated in the 39 and 78-micron mode for their MTF and DQE characteristics to ensure uniform performance. Further, perceptual studies will be conducted to compare system performance in both pixel modes for detection of small targets that are representative of microcalcifications. In addition, comparisons will be made to clinical mammography systems under identical experimental conditions.

The partnership has been very successful in many respects. It has enabled us to have direct input on various aspects of the design during the entire process rather than just expecting a deliverable. Several manuscripts are being submitted for publication on various aspects of this research, and design and performance of the detector.

ISSUES: The main issues relate to the inherent challenges in certain aspects of the development of the proposed devices. However, we have worked closely with our research partner company, Fairchild Imaging, and we believe we have overcome most of these obstacles. It has been a pleasure and a learning experience to have the opportunity to work closely with our partner company in this research and important results have been derived from this research partnership.

PI: Wolff Kirsch, M.D.
Loma Linda University
Neurosurgery Center for Research, Training, and Education
11175 Campus St., Suite 11113
Loma Linda, CA 92359
T: 909-558-7070
F: 909-558-0472
wkirsch@som.llu.edu

PROJECT TITLE: Iron Metabolism Alterations in Alzheimer's Disease

PARTNERS' NAMES AND AFFILIATIONS:
Daniel Collins, Ph.D. (BioE, Inc., St. Paul, MN), James Larsen, M.D., Daniel Kido, M.D., Barbara Holshouser, Ph.D., Andre Obenaus, Ph.D., William Britt, Ph.D., Floyd Petersen, MPH (Loma Linda University, Loma Linda, CA), Keith Coon, Ph.D. (Translational Genomics, Tempe, AZ), E. Mark Haacke, Ph.D. (MRI Institute for Biomedical Research, Detroit, MI)

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

ABSTRACT
Our BRP applies minimally invasive technologies to determine if altered brain iron metabolism in the face of Mild Cognitive Impairment (MCI) represents a significant risk factor for the development of Alzheimer's disease (AD). Goals are development of biomarkers for the diagnosis of AD based on a novel MRI imaging technology and peripheral blood assays (lymphocyte flow cytometry, genomic analysis) evaluating perturbed brain iron metabolism. Sequential MRI and blood studies are correlated with periodic psychometric evaluations of 75 MCI subjects and 25 normal controls over a 4 to 5 year period. The MR imaging technology will be validated by application to mice with an engineered deletion of the iron regulatory protein 2 (IRP-2) gene. This animal accumulates excessive amounts of brain iron and develops a neurodegenerative disorder. Direct assays of brain iron in its various forms ("free," transferrin bound, ferritin, non-heme, total) will be correlated with MRI signals from specific voxels and results extrapolated to human imaging studies. Dr. Tracey Rouault (NICHD), a consultant to the project, has developed this animal model and enabled us to develop the colony.
Inclusion and exclusion criteria for MCI and control subject recruitment have been defined with our project consultant, Dr. Ronald Petersen (Mayo Clinic). After screening with an interview, medical history, Mini-Mental score (Folstein) and Wechsler logical memory test, a consensus conference selects subjects for more detailed psychometric evaluation (Dr. Britt). After this study, subjects are assigned to the MCI, control group, or excluded. 991 subjects have been screened over the past 22 months and 50 selected as MCI and 26 controls. The selection process conforms to NIH regulated age, gender, and racial/ethnic considerations. Evaluators have obtained certification from the Washington University Alzheimer's Study Group to make Clinical Dementia Ratings (CDR). (Sequential CDR's are videotaped). Once selected, subjects have a yearly MRI and biannual blood and psychometric evaluations. The anticipated 15% annual conversion rate of MCI to AD is being experienced. A statistical data base has been constructed (Access) and implemented for storage, retrieval, and analysis with our statistician Floyd Petersen MPH.

STATUS OF RESEARCH AND PARTNERSHIP
Human Studies:
Special SWI imaging and spectroscopy: 63 subjects (41 MCI, 22 control) have had initial SWI baseline brain evaluations and spectroscopy. 13 subjects (3 controls, 10 MCI's) have had their second SWI and spectroscopic studies. Two control subjects have had a deteriorating neurologic course, CDR's going from 0 to 0.5 (MCI range). Sequencing parameters and protocols for SWI and MR spectroscopy have been standardized. In addition to single voxel spectral acquisitions in the posterior cingulate gyrus – a standard for MCI studies, a multi-voxel chemical shift acquisition technique has been designed with the Siemens Vision platform and is producing excellent spatial quality data. Dr. Kirsch and Mr. Petersen served as normal

volunteers to standardize the imaging protocols and select the appropriate scanners. Data acquisition for SWI imaging underwent several trials with difficult echo times (TEs) and flip angles before settling on a standard protocol with a TE = 40 mins. Serial hippocampal volume determinations are being made on each case.
Flow Cytometry: Our laboratory has prepared 5 different monoclonal antibodies (Mabs) to the iron degradation domain (IDD) of iron regulatory protein-2 (IRP-2). These reagents are utilized in our flow cytometric studies of the peripheral blood of our study subjects. An attempt to produce a Mab to the critical iron sensing nanopeptide of IDD was unsuccessful. Peripheral blood lymphocytes have been studied for quantitated Mab uptake – 59 having initial blood studies, 40 a second blood study, and 6 a third study. In view of the elevated IRP-2 levels noted in AD peripheral blood lymphocytes, and at the suggestion of Dr. Ronald Petersen (Mayo Clinic, our study consultant) 4 AD cases were studied. Two of the AD samples had remarkably elevated IRP-2 on Elisa testing and the cells were lysed, Western blots performed, with a positive identification of IRP-2. Human IRP-2 from AD lymphocytes is currently being sequenced by electrospray mass spectroscopy and compared to the control molecules.
Genomic Studies: In collaboration with Dr. Keith Coon, Translational Genomics Research Institute, annotation of the 63,138 bp of the human IRP-2 gene has been conducted with location of the critical nanopeptide cysteine IDD sequence in exon 5. Oligonucleotide primers have been constructed to all of the 22 IRP-2 exons. DNA sequence information was attempted on 50 formalin fixed AD occipital lobes, 50 control brains. Formalin fixed DNA was too degraded for assay, the study was repeated with fresh frozen brain tissues. DNA sequence information shows no significant polymorphisms in the AD or control IRP-2. Since we have detected no relevant germline mutation in the IRP-2 gene, we are hypothesizing that observed changes of increased expression of IRP-2 in AD cases could result from post-transcriptional mRNA modifications or splicing mistakes.

Animal Studies:

"Knockout" mice, SWI imaging, Iron assays: Our genetically engineered "knockout IRP-2" mouse colony is in production. A PCR assay has been developed to validate the gene deletion. The SWI imaging protocol (11.7T) has been perfected after initial high morbidity rates of mice in the coil by improved oxygenation and body warming. Iron assays for milligram quantities of brain have been established: "loosely-bound" iron, non-heme, and total iron are being measured. These assays will be correlated with SWI signals and extrapolated back to human studies.

ISSUES

Subject selection to meet MCI criteria and the NIH consensus for gender and ethnicity are stringent. On-site reviews and phone conference with our consultant Dr. Ronald Petersen (Mayo Clinic) has facilitated our selection process. All cases selected up to the time of Dr. Petersen's visit (41 cases, 15 MCI, 26 control) have been reviewed with attention to the statistical demands presented by the relatively small N and uncertainty inherent in our ultimate final diagnosis. In view of attrition in the elderly as well as diagnostic ambiguity we will increase our N (125 MCI, 30 control).
Initial imaging of normal mouse brain presented problems with full phantom and animal studies specific to the high field MRI (11.7T) as well as survival of the animal in the confined imaging tube. Software changes, revision of the SWI sequences for small animals, and improved anesthetic technique solved these problems.
Genomic studies on fixed tissue and blood were compromised by formalin degradation of DNA. We have subsequently switched to analysis of snap frozen specimens with successful sequencing. Genomic studies focus on exon 5 of the IRP-2 gene that codes for the critical iron sensing nonopeptide sequence of IRP-2. Our attempts to produce a monoclonal antibody to the nonopeptide, iron degradation domain of IRP-2 have been unsuccessful because of the high degrees of domain conservation in normal mice. IRP-2 "knockout" mice do raise a significant immune response to the critical iron sensing nonopeptide sequence and this avenue is being actively pursued to create a monoclonal antibody for flow cytometric application. Our NIH funding commenced October 1, 2002, so this report covers 21 months of work.

PI: Manfred R. Koller
Oncosis
6199 Cornerstone Ct.
Suite 111
San Diego, CA 92121
T: (858) 550-1770
F: (858) 550-1774
fkoller@oncosis.com

PROJECT TITLE:
Laser cell processing for basic and clinical research

PARTNERS' NAMES AND AFFILIATIONS:
James Leary
University of Texas – Medical Branch (Galveston, TX)

Manfred Koller
Cyntellect (San Diego, CA)

Robert Fischer
Optics-1 (Westlake Village, CA)

GRANTING NIH INSTITUTE/CENTER: NCRR

ABSTRACT
Numerous methods have been developed that rely on lasers to study and manipulate cells and tissues. Examples include inactivation of specific proteins or genes (e.g., chromophore-assisted laser inactivation), optoinjection of genes or macromolecules, activation of photosensitive agents (e.g., uncaging), photo-bleaching (e.g., motility/diffusion studies), and killing (e.g., cell purification). However, these potentially powerful techniques have been developed on low-throughput manual microscope systems, hampering their widespread use. To fully reap the benefits of these techniques, a novel automated imaging and laser-based processing technology for the analysis and manipulation of individual cells in a high-throughput manner has been developed. The technology, called the Laser-Enabled Analysis and Processing (LEAP™) platform, has a number of unique attributes. The LEAP platform incorporates a moveable stage for plate handling, LED and xenon lamp illumination and excitation, adjustable magnification (2.5X - 20X), dual CCD cameras for imaging in brightfield, darkfield, phase contrast, or multi-color fluorescence, a pulsed laser with galvanometer steering for rapidly manipulating individual cells, and software for image/data analysis and process control. The LEAP platform rapidly captures images of cells *in situ* (e.g., in a well plate) through a combination of galvanometer and stage motions, thereby limiting stage movement (and cell displacement), while still achieving throughputs over 100,000 cells/sec. This speed enables reading of cell-based assays (e.g., mitotic index, nuclear translocation, etc.) in 384 well plates in <5 minutes. If desired, the laser can be fired at individual cells with specified criteria to achieve various cellular manipulations. LEAP has many potential uses, and this proposal brings together several institutions and researchers to develop and investigate the possible applications of this novel technology.

STATUS OF RESEARCH AND PARTNERSHIP
The LEAP research platform has now been used in a variety of important applications by the partners including cell purification, cell transfection, and high-throughput high-content cell-based drug screening, thereby demonstrating the broad potential of the LEAP platform. For example, LEAP has been used in experiments to address the controversy surrounding the phenotype of the hematopoietic stem cell. In this

case, LEAP was used to detect and eliminate the 0.01 – 0.30% of $CD34^+$ cells that were left behind after two-pass flow cytometry sorting for $CD34^-$ cells, yielding a more accurate putative stem cell population that was used for in vivo stem cell assays. The more highly purified $CD34^-$ cells led to a different in vivo engraftment profile, indicating their more primitive status than $CD34^+$ cells. LEAP has also been used for *in situ* cloning of numerous adherent and non-adherent cell types. Most importantly, a method to clone cells based on *in situ* measurement of individual antibody secretion rate was developed, resulting in generation of cell lines with potential for greatly improved biopharmaceutical manufacturing yields. Further, LEAP was used to develop and implement RNA interference (RNAi)-mediated gene silencing (via optoinjection) in several cell-based functional genomics applications. Both direct RNA-mediated and indirect DNA-mediated (coding for siRNA) RNAi have been demonstrated, with results indicating that optoinjection has advantages over other transfection techniques with respect to simplicity, high cell viability, speed, and selectivity. This unique combination of capabilities makes LEAP a powerful new analysis and laser-based cell manipulation technology with many potential applications.

ISSUES

The BRP is now up for renewal, but NCRR will no longer support these awards.

PI: Simon P. Levine, Ph.D.
Department of Physical Medicine and Rehabilitation and
Department of Biomedical Engineering.
1C335 University Hospital
1500 E. Medical Center Dr.
Ann Arbor, MI 48109-0032
T: 734-936-7170
silevine@umich.edu

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

PARTNERS' NAMES AND AFFILIATIONS:
The University of Michigan (Ann Arbor, Michigan)
Henry Ford Hospital (Detroit, Michigan)
Technical University Graz (Graz, Austria)

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

ABSTRACT
The University of Michigan Direct Brain Interface (UM-DBI) research partnership is a collaboration which includes the Departments of Biomedical Engineering, Electrical Engineering and Computer Science, Physical Medicine and Rehabilitation, Neurology, Surgery and Radiology from the University of Michigan; the Department of Neurology from the Henry Ford Hospital, and the Institute of Biomedical Engineering from the Technical University Graz. This partnership is working to address the functional evaluation of a direct brain interface and the optimization of detection methods used in the direct brain interface.
The functional evaluation includes real-time, on-line implementation and testing with subjects at the University of Michigan and Henry Ford Hospitals who have implanted electrodes for purposes related to epilepsy surgery. (While these subjects are not members of the target user population, the presence of implanted cortical electrodes in these subjects provides a unique opportunity for direct brain interface development). Specific activities include: 1) Development of an on-line, real-time testing system for direct brain interface methods; 2) Examination of the ability of subjects to learn to voluntarily improve event-related potential (ERP) quality and detection performance given appropriate feedback; 3) Determination of the accuracy and speed with which a direct brain interface can be used to perform functional tasks; and 4) Identification of the relationship between the location of electrocorticogram (ECoG) recorded brain events and the activated portion of the brain as observed through functional magnetic resonance imaging.
Improvements in the accuracy by which brain events can be detected are being approached through development and optimization of time-domain based detection methods and evaluation of the performance of frequency-domain based detection methods on ECoG. In addition, off-line analysis is being used to 1) Investigate the ability of current detection methods to differentiate between brain activity related to different actions and 2) Determine the increased accuracy of event detection achievable using ECoG versus EEG.

STATUS OF RESEARCH AND PARTNERSHIP

Real time system development (University of Michigan)

The real-time direct brain interface (DBI) test system is under continuous refinement to allow more flexible testing. A new version of the software that incorporates frequency based features for improved detection accuracy and a quicker response time is undergoing final tests.

On-line direct brain interface testing and time-based detection (University of Michigan and Henry Ford Hospital Subcontract)

Experiments examining the ability of subjects to produce improved direct brain interface operation by voluntarily altering electrocortical brain activity (ECoG) given appropriate feedback and determining the accuracy and speed with which a DBI can be used to perform a functional task have been performed with 6 subjects so far this year. Subjects completed one or more template collection blocks of approximately 50 repetitions of an action without feedback to establish an ERP template. Subjects then completed 2 to 5 feedback blocks of 50 repetitions during a 2-3 hour data collection session. The most recent subject also performed feedback blocks using imagined tongue movements instead of actual tongue movements with comparable online detection accuracy.

Correlation of fMRI and ECOG *(University of Michigan)*

Comparison, through functional magnetic resonance imaging (fMRI), of event-related potential (ERP) locations to fMRI activated regions for the same tasks are fully underway. Data has been collected from seven subjects (two epilepsy patients with prior ECoG experiment participation and five controls) and the efficacy of imagined movements to produce statistically significant event-related activation has been verified. Both qualitative and quantitative comparison of the locations of fMRI activation and good recording channels (from studies conducted on the epilepsy patients) show a high level of agreement. For the purpose of quantitative comparison, a correlation between the T-statistic (of voxels under consideration) obtained from fMRI studies and the corresponding detection accuracy of the ECoG electrodes will be determined. Further experiments to study the predictability of locations of good ECoG channels from fMRI data are underway.

Development of Improved Detection Methods *(University of Michigan and Graz subcontract)*

The development and evaluation of improved single-channel detection methods continues. Offline experiments with the developed algorithms (autoregressive (AR), wavelet (WAV), and cross-correlation template matching (CCTM)) showed two issues important to online experiments. First, the analysis delay inherent in the methods needs to be reduced for effective use in feedback experiments. Second, the generalization of the training results to the testing data needs to be verified with an appropriate validation.

Under the Graz subcontract a new detection approach based on wavelet methods (WAV) was developed. Initial offline testing indicated dramatic improvements in detection accuracy and the potential for decreasing feedback delay. (Based on these results an Administrative Supplement was granted to continue the Graz sub-contract beyond its initial term of 3 years through years 4 and 5 of the grant).

Offline evaluation of the WAV method showed that use of a symlet wavelet in combination with the threshold detector did not perform very well with reduced delays, therefore other features including different wavelets and adaptive autoregressive parameters were investigated. First results showed that the generalization could be increased and the detection delay reduced while still yielding good detection performances. A comprehensive analysis of 2184 ECoG channels is underway to quantify the overall performance of the improved method.

In parallel, work has been done to substitute a statistical classifier for the simple threshold detector. By using a classifier not only should brain patterns associated with movement imagery be detected, but it should also be possible to discriminate between different brain patterns, extending the simple brain switch to a multi-class direct brain interface.

ISSUES:

No Input

PI: Klaus Ley, PhD.
Department of Biomedical Engineering
University of Virginia
T: (434) 243-9966
klausley@virginia.edu

PROJECT TITLE: Biomechanics of Leukocyte Adhesion Molecules

PARTNERS' NAMES AND AFFILIATIONS:
Michael B. Lawrence, University of Virginia
William H. Guilford, University of Virginia
Jonathan R. Lindner, University of Virginia
Geoffrey S. Kansas, Northwestern University

GRANTING NIH INSTITUTE/CENTER: NIBIB, formerly NHLBI

ABSTRACT
This BRP was initiated to conduct interdisciplinary bioengineering research in the area of molecular biomechanics. Leukocyte and endothelial adhesion molecules govern the trafficking of cells in inflammation, immunity, cancer metastasis and other processes. Some adhesion molecules, among them the selectins, are specialized to mediate adhesion in the presence of blood flow. Pressure-driven blood flow is associated with a shear stress exerted on the vessel wall, which results in a force on leukocytes and other cells trying to adhere to the endothelium. It is believed that adhesion under shear stress requires adhesion molecules with rapid association rates (on-rates), resulting in rapid formation of bonds. In vitro experiments and modeling studies indicate that the selectins also have high rates of bond dissociation (off-rates). Preliminary data suggest that the off-rates of selectins vary systematically with the shearing force exerted on the cell bound by the selectin (reactive compliance or tensile strength). In addition, the release of at least one of the selectins is accelerated by proteolytic cleavage by a surface-bound or membrane integral metalloproteinase. The BRP has four specific aims. (1) To measure the bond lifetimes and apparent off-rates of L-, P- and E-selectin bound to their natural ligands. (2) To determine the role of L-selectin shedding in regulating leukocyte adhesion and selectin kinetics. (3) To determine the impact of the selectin length and their cytoplasmic tail for the biomechanics of adhesion under shear flow. (4) To design and build beads, liposomes and gas-filled bubbles (ultrasound contrast agents) that use leukocyte adhesion molecules to bind to vessel walls under shear stress. Each of these aims is approached in a three-pronged fashion. We propose to use laser trapping technology to directly measure biomechanical and kinetic parameters of selectin bonds, use single cells on sparse substrates to understand the biomechanics of selectins in an in vitro flow chamber, and use intravital microscopy to study selectin biomechanics in the context of the living organism. We use molecular biology techniques to manipulate cDNA, cells, and mice to isolate each molecular mechanism. The insights gained from basic science-oriented studies are used to design liposome-based targeted drug delivery systems and ultrasound contrast microbubbles for delivery in the vascular system under shear flow.

STATUS OF RESEARCH AND PARTNERSHIP
The partnership is in active progress. We exchange reagents (antibodies, transfected cell lines) and technical expertise. The application of adhesion molecules for targeting purposes has been particularly successful. Dr. Lindner has successfully imaged metastatic tumors, atherosclerotic plaques, inflammation and angiogenesis by targeted ultrasound contrast in mice. We have organized four Colloquia on the Biomechanics of Adhesion Molecules in 2000, 2001, 2002 and 2003. Each was attended by approximately 50 scientists and graduate students each. We have recently discovered a way to greatly enhance targeting of microbubbles for molecular imaging. This was based on discoveries in Dr. Lawrence's and Ley's labs. Other areas are also progressing very well, with a very exciting paper coming

out of the laser trap work which allows detailed comparisons of the adhesion strength of single E- and P-selectin molecules under controlled rates of loading.
We have continued to use intravital microscopy to investigate the effect of varying shear rate and shear stress on leukocyte rolling. We found that leukocyte rolling velocity is not only controlled by selectins, but also by integrin binding, more specifically, by LFA-1 and Mac-1. During this rolling interaction, which also requires selectins, neutrophil Mac-1 appears to bind to endothelial ICAM-1, whereas LFA-1 binds another, unknown ligand. We have now firmly established that rolling velocity is invariant when plasma viscosity is increased twofold, and that this is due to cellular, not molecular properties. Beads coated with selectin ligands do not behave that way, but show the expected increase of rolling velocity with increased shear rate. One factor in this process is cellular deformation and the other, possibly related factor is tether formation as shown by Michael Lawrence's group last year.
Based on our intravital microscopic experience, we have developed a new autoperfused flow chamber. This flow chamber allows to study the interaction of unadulterated blood cells with defined substrates, such as selectins, ICAM-1 or VCAM-1. This approach obviates the need for blood cell isolation, which can lead to inadvertent activation, and allows flow chamber experiments with very small perfusion volumes, thus making possible, for the first time, flow chamber studies using mouse blood. Initial studies have revealed an unexpected synergism between P- and E-selectin, and we plan to use this novel flow chamber and variations thereof for studies into biomechanics of leukocyte rolling and adhesion. It is already clear from preliminary experiments that the autoperfused flow chamber will be invaluable in determining molecular mechanisms of synergy between different adhesion molecules.
The applied aim 4 continues to be very successful and advance rapidly. In an in vitro system, we have carefully characterized the properties of targeted microbubbles during adhesion under flow and were able to control cell adhesion using engineered interfaces with spatially varying gradients. Microparticle adhesion to P-selectin depends on the length of the ligand-bearing molecules. These insights were used to further optimize the delivery of ultrasound contrast agents to sites of inflammation, ischemia and angiogenesis. We have prepared such contrast agents with novel surface features and filed two patent applications.
Using microbubbles conjugated to echistatin, a peptide binding to $\alpha v\beta 3$ integrin, Dr. Lindner was able to image angiogenesis in vivo. An exciting application that reaches beyond the cardiovascular area is the successful imaging of tumor angiogenesis in vivo, using molecular imaging targeted to $\alpha v\beta 3$ integrin, which is preferentially expressed on growing microvessels. These ultrasound-based targeting strategies will clearly be clinically useful for diagnostic purposes in cardiology, inflammatory diseases and cancer. Beyond diagnostics, we were able to use this technology to transfect endothelial and parenchymal cells with an experimental plasmid. This provides a proof-of-principle for targeted gene therapy using molecular imaging to precisely guide the area of transfection.

ISSUES
None.

PI: Shu-Tung Li, Ph.D.
Collagen Matrix, Inc.
509 Commerce Street
Franklin Lakes, New Jersey, 07417
T: (201) 405-1477
F: (201) 405-1355
sli@collagenmatrix.com
www.collagenmatrix.com

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

PARTNERS' NAMES AND AFFILIATIONS:
Frank Liuzzi, Ph.D. (University of South Florida, Tampa, FL) (USF)
Roger Madison, Ph.D. (Duke University, Durham, NC)

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

ABSTRACT
The goal of this project is to design, engineer and evaluate *in vivo* a type I collagen-based nerve guide for peripheral nerve regeneration applications. The objectives of this project entail the isolation of the pertinent design parameters necessary for the initial prototype screening in a rat sciatic nerve model. The final prototype, engineered from optimal design parameters, will be evaluated in primates as a potential entubulation repair method for clinical applications. The key design parameters that have been investigated include permeability of the nerve guide membrane, axonal growth guiding channels, cell growth inductive and cell adhesive properties and structural stability. This report covers the progress made over the past three years.

STATUS OF RESEARCH AND PARTNERSHIP
The rat sciatic nerve screening studies evaluating the permeability and guiding channels have been completed at USF. We have initiated studies with nerve guides containing axonal growth promoting molecules (bFGF and IGF-II) and a cell adhesive molecule (laminin) at USF. We have also initiated a study to evaluate the structural stability and the rate of *in vivo* resorption of the nerve guides at the University of Arizona. Training of primates continues at Duke University in anticipation of nerve guide implantations in December of 2004. The details of the studies are described below.

Permeability: Repair of a 1 cm rat sciatic nerve gap has been completed using nerve guides having two different permeability properties defined as large pore nerve guides (LPNG) (MW cut-off ~ 270 kDa) and small pore nerve guides (SPNG) (MW cut-off ~ 16 kDa). Animals were sacrificed at 6 and 12 weeks. Histomorphometric measurements included counting 100 % of the myelinated axons, measuring ~10 % of the myelinated axon diameters and determining the area occupied by the myelinated axons from the mid-sections of the repaired nerves and from the contralateral sciatic nerves. The results showed no statistically significant differences in the number of myelinated axons at 6 and 12 weeks post-implantation between the LPNG and the SPNG; however, a statistically significant difference was found between the average diameter of axons of the LPNG and SPNG at 12 weeks (SPNG > LPNG, $P = 0.026$) and in the area occupied by axons of only the SPNG between 6 and 12 weeks (12 wk > 6 wk, $P = 0.024$). The Sciatic Nerve Function Index (SFI) could not be accurately determined due to the poor quality of the walking tracks. Electrophysiological methods were evaluated and optimized in this study for determining the compound muscle action potential.

Guiding Channels: The rat study has been completed using nerve guides containing either micro-tubes or filaments as axonal guiding channels. The data is currently being evaluated. Initial findings based on the number of regenerated myelinated axons after 12 weeks of implantation indicated the following order: autograft > nerve guide with micro-tubes ~ empty nerve guide > nerve guide with filaments. Signifcantly, the micro-tubes provided an improved structural support for the axonal growth over the empty nerve guides.

Inductive and Adhesive Molecules: The rat studies have been initiated using nerve guides containing either bFGF or IGF-II, or micro-tubes containing laminin. In anticipation of accelerated axonal regrowth due to the presence of the bioactive molecules, these studies were designed only for 6 weeks. Animals have been sacrificed and data is currently being collected and evaluated.

Effects of Sterilization on the Activity of the Bioactive Molecules: In the development of a bioactive nerve guide, the effect of sterilization on the activity of the bioactive molecules must be considered. Nerve guides containing bFGF, IGF-II and laminin were sterilized by γ-irradiation at 16 kGy on dry ice to minimize the structural damage. Cell proliferation assays were conducted to determine the activity of the growth factors. Nerve guides containing bFGF were cultured on BHK-21 and PC-12 cells and nerve guides containing IGF-II were cultured on PC-12 cells. Non-sterile samples served as controls. Results showed that there was not a significant loss in activity upon γ-irradiation. This is a significant improvement over previous sterilization conditions where a 50% loss in activity was observed upon γ-irradiation at 25 kGy at room temperature. Results of a qualitative organ culture study measuring the neurite outgrowth from rat embryonic spinal chord explants showed that laminin remained active after sterilization. More quantitative neurite outgrowth experiments using PC-12 cells are on-going.

In Vivo Resorption Studies: A rat subcutaneous implantation study is currently being conducted to evaluate the structural stability and the rate of resorption of the nerve guide *in vivo*. Nerve guides, with and without micro-tubes, have been implanted and will be explanted after 6, 12 and 24 weeks post-surgery. Since the rate of resorption is directly correlated with the hydrothermal stability, nerve guides having two distinct hydrothermal shrinkage temperatures (55-65°C and 65-75°C) were implanted. Explants will be evaluated histologically to determine the extent of collagen resorption as well as the structural integrity of the nerve guides at the specified time points.

Primate Studies: Training of four monkeys over the past 2 ½ years have resulted in the collection of consistent baseline data for measuring the key grip strength in preparation for the median nerve repair study. Extensive reversible nerve block experiments have been conducted on these monkeys to ensure that a median nerve lesion at the wrist would result in a detectable deficit in the behavioral study. Each monkey will serve as its own control, having the identical nerve gap length repaired with an autograft on the contralateral side. Additional monkeys will be procured and trained in preparation of the nerve guide implantation in December 2004.

ISSUES

Inter-institutional communication and support have been an on-going concern. Much effort has been devoted to improve timely feedback from the partners regarding technical issues, study protocol reviews, data collection and analysis as well as scheduling of various studies. Some of these issues have resulted in loss of productivity.

PI: CC Ling, Ph.D.
Memorial Sloan Kettering Cancer Center
1275 York Avenue,
New York, NY, 10021
T 212 639 8301
lingcc@mskcc.org

PROJECT TITLE: Multimodality Biological Imaging of Cancer/Tumor Hypoxia

PARTNERS' NAMES AND AFFILIATION:
JD Chapman, RF Schneider, Fox Chase Cancer Center, Philadelphia, PA
M Welch, Washington University, St Louis MO
LE Gerweck, Massachusetts General Hospital, Boston MA
J Russell, Memorial Sloan Kettering Cancer Center, New York NY

GRANTING NIH INSTITUTE/CENTER: Biomedical Imaging Program, NCI, NIH

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

STATUS OF RESEARCH AND PARTNERSHIP
Using two recently acquired small animal magnetic resonance (MR) imaging units (field strengths 4.7 and 7.0 Tesla, bore diameters 310 and 400 mm) and a dedicated coil built in-house, we have improved the signal to noise ratio compared to the standard built-in scanner coils. We have performed high resolution imaging (90□m x 90 □m x 200□m) on tumors in rats. This will allow us to detect areas of necrosis, and remove them from the analysis when we use PET and immunohistochemistry to correlate hypoxia and blood flow.

To detect tumor hypoxia, we have used three radiotracers, iodine-124 labeled iodo-azomycin galactoside (^{124}I-IAZG), fluorine-18 labeled fluoromisonidazole (^{18}F-FMISO), and copper-64 labeled diacetyl-bis (N4-Methyl thiosemicarbazone (^{64}Cu-ATSM). Serial microPET imaging was used to evaluate ^{124}I-IAZG as a hypoxia imaging agent in tumor-bearing mice and rats, the larger tumor sizes in rats allowing more definitive evaluation of the intra-tumoral distributions of these tracers. For comparison, ^{18}F-FMISO and the tumor-imaging agent fluorine-18 labeled fluoro-deoxyglucose (^{18}F-FDG) were also imaged in the same tumor-bearing animals. We also directly measured tumor oxygenation with the Oxylite™ probe system, and found that tumor size can be used as an index of hypoxic fraction (HF) in these xenograft models. We therefore classify large tumors (~700-800 mg) as having high-HF and those of ~200 mg or less as low-HF tumors.

Both ^{124}I-IAZG and ^{18}F-FMISO showed high-contrast uptake in large, high-HF but not in small, low-HF tumors. However, due to differences in isotope half-life, ^{18}F-FMISO imaging must be performed considerably earlier than ^{124}I-IAZGP and consequently the optimal ^{124}I-IAZG images (at 24-48 hr post-injection (p-i)) have higher contrast than the best ^{18}F-FMISO images (up to 8 hr p-i). Region-of-interest analysis and tissue counting ex vivo corroborated this conclusion. The decay-corrected ^{18}F-FMISO activity concentration (% of the injected dose per gram of tissue, %ID/gm) at 3 hr p-i is 10- to 15-fold higher than that for ^{124}I-IAZG. However, in terms of the tumor-to-normal tissue ratio, the value is higher for ^{124}I-IAZG than ^{18}F-FMISO for most tissues in the animals with the large tumors both in mice and rats, showing the clinical potential of ^{124}I-IAZG for non-invasively identifying tumor hypoxia.

We have also evaluated the hypoxic tracer, ^{64}Cu-ATSM. Using four cell lines of human origin and 2 rodent cell lines, we have shown that ^{64}Cu accumulation is rapid during the first 0.5-1 hr of incubation, after which it may increase slightly, or decrease, depending on the cell line and the pO_2. Uptake is highest in anoxic cells; when cells are made hypoxic (0.5% O_2) their uptake is slightly greater than in normoxia, suggesting that Cu-ATSM may be less suited for the identification of intermediately hypoxic tumor regions, but could be a good marker for extreme hypoxia/anoxia. The extent of ^{64}Cu-ATSM uptake in anoxia was also cell line dependent; in a panel of six lines, all made anoxic, the highest accumulating line took up more than twice the activity of the lowest line.

^{64}Cu-ATSM uptake was studied in vivo using microPET and comparing it to ^{18}F-FMISO. Rats bearing either FaDu or R3327AT tumors received ^{18}F-FMISO. They were then imaged between 1 and 4 hr p-i , given ^{64}Cu-ATSM and imaged 1-2 hours p-i and again at 16- 24 hours p-i. The distribution of ^{18}F-FMISO in both tumor types tended to become more focal at longer times. For ^{64}Cu-ATSM, the intratumoral distribution in FaDu tumors did not change with time. However for the R3327-AT tumors, the intratumoral distribution "flipped" so that regions that were relatively hot at early times became relatively cold at later times and vice-versa.

Image datasets were aligned in three-dimensions using software developed in-house and regions of interest (ROI) drawn around the tumor simultaneously in all aligned images. The voxel values from the ROIs were plotted as scattergrams. In agreement with our subjective assessment of the images, there was a high degree of correlation between ^{18}F and ^{64}Cu images for FaDu tumors regardless of when the ^{64}Cu images were obtained. For the R3327AT tumor there was a good correlation between ^{18}F-FMISO and late ^{64}Cu-ATSM, but predominantly low or even negative correlations between ^{18}F-FMISO and early ^{64}Cu-ATSM and between early and late ^{64}Cu-ATSM. Direct probe measurements of tumor hypoxia showed that enhanced uptake of ^{64}Cu-ATSM at later times p-i corresponded approximately to regions of low pO_2 whereas regions of enhanced uptake of ^{64}Cu-ATSM at early times p-i did not. These data indicate that ^{64}Cu-ATSM did not consistently detect tumor hypoxia and that the use of ^{64}Cu-ATSM to spatially define tumor hypoxia in order to prescribe radiation dose distributions should be approached with caution. It also demonstrates that nuclear medicine images may need to be co-registered for proper interpretation.

We are now evaluating a novel approach for hypoxia imaging, based on hypoxia inducible factor-1 (HIF1) driven expression of a viral thymidine kinase/green fluorescent protein fusion protein (HSVTK/GFP). This allows for PET imaging of gene activity through the thymidine kinase substrate ^{124}I labeled 2'-fluoro-2'-deoxy-5-iodo-1-beta-d-arabinofuranosyluracil (^{124}I-FIAU); gene activity can also be visualized microscopically with green fluorescent protein. This construct has been retrovirally transduced into R3327AT cells. In vivo experiments, using *phosphor plate imaging* were performed to compare the spatial distribution of ^{124}I-FIAU *and ^{18}F-FMISO in xenografts. The data* indicate that ^{124}I-FIAU accumulation, resulting from the HSVTK/GFP gene expression, occurs in those areas identified by ^{18}F-FMISO uptake as being hypoxic. Similar results were obtained in studies using an animal positron emission tomography (PET) unit. Thus, this cis-reporter system is sufficiently sensitive to detect hypoxia-induced transcriptional activation by non-invasive imaging and might provide a valuable tool in studying and monitoring tumor hypoxia during the development and treatment of rodent tumor models.

Clinical PET images show that the distribution of the metabolism tracer FDG is frequently non-uniform within tumors. In order to understand the possible causes of this heterogeneity, we studied FDG microdistribution in R3327-AT tumors in nude mice. Four R3327-AT tumor bearing mice were co-injected with ^{18}F-FDG and pimonidazole (a hypoxia marker) at 1 hr prior to sacrifice. At 40 and 20 minutes prior to sacrifice, the mice were injected intra-peritoneally with bromodeoxyuridine (BrUdR) which is taken up by S-phase cells. One minute prior to sacrifice, the mice were injected with Hoechst-33342, a fluorescent dye, which serves as a blood flow marker. Marker distributions were acquired by autoradiography and fluorescent microscopy. The images were first co-registered and resolution of the microscopic images was degraded to match that of autoradiography. The statistical analysis of each data set was then performed on the pixel-by-pixel basis. The necrotic regions of the tumors as well as stromal tissue were masked out and excluded from the analysis.

Statistical analysis of the data demonstrated a positive pairwise correlation between FDG uptake and pimonidazole in each of the four tumors ($p < 0.01$). and a negative correlation between FDG uptake and perfusion. Multiple regression analysis using 3 independent variables (intensities of Hoechst 33342, pimonidazole and BrdU stains) demonstrated that the intensity of pimonidazole staining was the best predictor of FDG uptake. In conclusion, this study showed that in this prostate carcinoma tumor model, hypoxia is the major determinant of increased FDG uptake, suggesting some up-regulation of glucose metabolism in the regions of hypoxia.

ISSUES

We have still to apply our template system to co-registering NMR and PET data; knowing the location of the tip of the oxygen electrode is complicated by the probes tendency to bend in response to tissue resistance; the possibility that inserting the probe disrupts tissue blood supply to such an extent that it interferes with tumor oxygenation is being investigated by MRI.

PI: Frederic Lizzi, ENG.SC.D.
Riverside Research Institute
Biomedical Engineering Laboratories
156 William Street
New York, NY 10038
T: 212-502-1774
F: 212-502-1729
lizzi@rrinyc.org
www.rri-usa.org

PROJECT TITLE: Integrated Ultrasonic Systems for Non-invasive Therapy

PARTNERS: NAMES AND AFFILIATIONS:
Weill Medical College of Cornell University (New York, NY)
Columbia University College of Physicians & Surgeons (New York, NY)
Spectrasonics Imaging, Inc. (Wayne, PA)

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

ABSTRACT
The ultimate objective of this 5-year Biomedical Research Partnership (BRP) is to develop a unified body of scientific knowledge and validated technology concepts that are needed to establish ultrasound as a practical non-invasive treatment modality and to inaugurate ultrasonic therapeutics as a new biomedical discipline. The program systematically elucidates the spectrum of ultrasound therapeutic lesions that can modify various classes of diseased tissues. It develops integrated ultrasonic systems to position, induce, and monitor these lesions. The research is focused on establishing a comprehensive basis for future 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.

This Biomedical Research Partnership involves: biomedical engineering research at Riverside Research Institute (RRI); animal research studies at Weill Medical College of Cornell University (WMC) and Columbia University College of Physicians and Surgeons (CUCPS); and, advanced systems development at Spectrasonics Imaging, Inc. (SSI). Our multi-disciplinary research is designed to achieve a series of fundamental advances in the diverse areas involved in therapeutic ultrasound. It employs extensive theoretical modeling to elucidate physical ultrasound-tissue interactions that can be used to produce therapeutic changes in diseased tissues. The research validates model results for thermal and mechanical effects in a series of animal experiments. Validated results are used to design and implement advanced therapy systems incorporating ultrasonic arrays and real-time lesion monitoring. The systems are being tested and refined using animal experiments that investigate cancer and heart-disease therapy.

Our results will be incorporated in a systems model of ultrasonic therapy which will permit comprehensive treatment planning and design of future system features.

STATUS OF RESEARCH AND PARTNERSHIP
The BRP is now entering its fourth year and progress is being made on a number of interrelated issues, using working relationships that have been established among all partners. Significant progress has been achieved in: systems development; lesion modeling; tissue property measurements; and, lesion experimentation.

In terms of system development, we designed, implemented, and tested a versatile system that integrates, controls, and monitors high-intensity focused ultrasound (HIFU) exposures designed to

produce therapeutic lesions by, primarily, thermal phenomena. The system is used with custom HIFU arrays that contain central diagnostic arrays. The diagnostic and visualization functions are performed using a subsystem that provides full digital control over high-resolution diagnostic ultrasound arrays. Custom software controls all aspects of the imaging (e.g., electronic focusing, frame rates, etc.) with a graphical user interface (GUI). The HIFU transducer is excited using a 16-channel power amplifier controlled by a digital sub-system and waveform synthesizer. Desired HIFU exposure parameters (apodization, frequency, time duration, focal length, intensity) are specified through a GUI. The GUI also permits the operator to specify a variety of synchronous excitation modes for the HIFU and diagnostic arrays.

The system has been employed with three types of transducers developed in the program. These employ spherical-cap HIFU transducers with a 5-MHz center frequency, a 33-mm outer diameter, a 35-mm geometric focus, and a central aperture housing the diagnostic array. The first transducer type is a single-element cap with a fixed 35-mm focus. The second is a five-element annular array whose focus can be electronically varied between 30 and 40 mm. The third is a five-element array whose electrodes define parallel strips that broaden the focused beam's cross-section along the cross-strip direction and, therefore, produce larger therapeutic lesions. The diagnostic linear array supports dynamic focusing and can be operated at center frequencies of 7.5 MHz. The system has been successfully tested and calibrated using each transducer type.

Special operational modes have also been successfully tested. The first mode intersperses conventional diagnostic imaging at pre-selected intervals during HIFU exposures: this mode provides accurate positioning over the target area and permits B-mode monitoring during treatment. In addition, radio-frequency echo signals are digitally acquired for more complete assays of microstructure changes using ultrasonic spectrum analysis. The second mode uses radiation force elastography to monitor stiffness changes as HIFU lesions are formed. This mode applies a short, sub-threshold HIFU exposure to induce local tissue motion *via* radiation force. The motion is tracked with the diagnostic transducer to derive an index of tissue stiffness. Data are obtained before, during, and after HIFU therapy exposures to sense stiffness increases that accompany lesion formation. The third mode launches a brief sub-threshold pulse from the HIFU transducer and receives resulting tissue echoes using the diagnostic array. This harmonic-imaging mode senses non-linear propagation arising from high HIFU pressure amplitudes, and detects resultant harmonics, which affect absorbed doses. It also detects treatment-induced changes in the tissue non-linearity parameter, and it senses bubble formation, which can interfere with HIFU beam propagation.

A comprehensive theoretical model is being developed to better understand the physical phenomena involved in lesion production and monitoring. The model accounts for diffractive beam formation, non-linear propagation, acoustic refraction, tissue absorption and heating, and lesion production, using a thermal dose formulation. In addition, a model has been developed to describe radiation-force motion used to assay tissue stiffness changes.

Several series of tissue experiments have been conducted. Measurements have been made of acoustic properties in *in-vitro* cardiac and liver specimens to accurately apply our models and to better select HIFU exposures for optimal lesion production. *In-vitro* studies of bovine cardiac tissues verified system performance and showed excellent agreements with our models in terms of the size, shape, and severity of induced lesions. These experiments also documented the effects of exposure parameters on lesion attributes. Results have been used to plan *in-vivo* experiments in open chest dogs. Initial *in-vivo* experiments have demonstrated an ability to produce desired HIFU lesions; these are now being expanded to optimize exposure strategies and to document chronic cardiac effects. *In-vitro* and *in-vivo* studies in liver and other tissues have provided information relevant to tumor therapy and have tested our radiation-force and harmonic monitoring modes. These studies are now being expanded to treat *in-vivo* animal tumors in rats and rabbits.

ISSUES

None.

PI: Gerald E. Loeb, M.D.
University of Southern California
A.E. Mann Institute for Biomedical Engineering
1042 W. 36th Place
Los Angeles, CA 90089-1112
T: (213) 821-1112
F: (213) 821-1120
gloeb@usc.edu
http://www.usc.edu/dept/biomed/faculty/loeb.html

PROJECT TITLE: BION Treatment of Neuromuscular Dysfunction

PARTNERS' NAMES AND AFFILIATIONS:
Frances J.R. Richmond, PhD. A.E. Mann Institute – University of Southern California
Lucinda Baker, PhD PT, Biokinesiology and Physical Therapy, University of Southern California
Carolee Winstein, PhD PT, Biokinesiology and Physical Therapy, University of Southern California
Robert L. Waters, MD, Rancho Los Amigos National Rehabilitation Center

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

ABSTRACT
In theory, a wide range of sensory and motor dysfunctions can be treated by electrical stimulation to evoke patterns of neural activity similar to those that underlie normal function. In practice, however, such stimulation has typically required relatively expensive and large devices implanted by a surgeon or skin surface stimulation applied by a trained therapist. We have developed a 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 develop and test a range of clinical applications to determine safety and efficacy and to further understand the mechanisms underlying neuromuscular pathology and treatment. In the first five years, these applications include activating and strengthening muscles in the shoulder, forearm and hand in patients suffering from stroke to reverse shoulder subluxation, minimize hand contractures and strengthen hand muscles to assist with constrain-induced therapy. 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 (BION2S 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

To date, six patients have completed the Shoulder subluxation protocol (4 randomized to the surface stimulation group). An additional three patients have completed the Wrist contracture study. Subjectively, two of the three patients receiving BION treatment for wrist contracture regained voluntary control of wrist extension though they remained dependent on BION activation for finger flexion. A pre-BION clinical investigation for the wrist facilitation study to determine the parameters for surface EMG-triggered BION activation of functional wrist movement requiring wrist and finger flexion was completed. The FDA-IDE and IRB applications for EMG-triggered wrist facilitation during constrain induced therapy are in process with the trial start date planned for December of 2004.

The preclinical feasibility studies for BION treatment of pressure sores and obstructive sleep apnea include design development of embedded BION external hardware that provide power and communication to implanted BIONs in wheel chair back support, bed pads and pillows. Engineering test results of the first iteration BION1-2 (formerly BION2S) ASIC, a direct replacement for the BION1 ASIC, revealed a decoding error for some of the range of pulsewidth. A new version with the simple correction is expected back from the foundry by the middle of July. Deployment of the BION 1-2 for clinical trials is scheduled to take place by 10/2004. Replacement of the BION1 with the BION1-2 does not require any significant changes in the electronic subassembly or glass encapsulation. We expect regulatory approval as a minor amendment to the protocols.

ISSUES

No major administrative issues were encountered during the current funding. However, clinical trials were suspended for a four-month period due to non-adverse BION failures. Risk analysis was performed; new production tests to further minimize the possibility of BION failures have been implemented. In addition, the minor design flaw responsible for these failures has been corrected and implemented in the current BION 1-2 production run. BION1 and BION 1-2 production during the next funding period will be sufficient to meet the projected needs of the three NIH-funded clinical trials, two Canadian clinical trials and an additional two clinical studies of other potential BION applications. For example, pre-clinical feasibility studies and pre-BION clinical studies for the use of BION therapy for the prevention of pressure sores in spinal cord injured subjects and obstructive sleep apnea are continuing.

PI: Richard G. Long
Department of Blindness and Low Vision Studies
Western Michigan University
1903 West Michigan Avenue
Kalamazoo, Michigan 49008-5218
T: (269) 387-3451
richard.long@wmich.edu

PROJECT TITLE: Blind Pedestrians' Access to Complex Intersections

PARTNERS' NAMES AND AFFILIATIONS: Boston College, Boston, MA; Maryland School for the Blind, Baltimore, MD; University of North Carolina Highway Safety Research Center, Chapel Hill, NC; North Carolina State University, Institute for Transportation Research and Education, Raleigh, NC; Vanderbilt University, Nashville, TN

GRANTING NIH INSTITUTE/CENTER: National Eye Institute

ABSTRACT
The goals of this partnership are to identify street-crossing problems experienced by pedestrians with blindness and visual impairment 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

The work of the Western Michigan team focused on completing our study of blind pedestrians' access to the Nashville Music Row roundabout. In light of the anticipated shift in Year 5 from problem identification research to intervention research at roundabouts, WMU also led the effort to develop new methods for evaluating the effectiveness of interventions to enhance crossing safety. WMU research staff will lead an investigation this fall at roundabout located in Raleigh, NC to test a device that alerts blind individuals that drivers have yielded for them. Regarding the engineering development of the Anti-Veering Training (AVTD) technology, the team is well into the final stages of manufacturing 15 AVTD's (anti-veering training devices). Complete components sets (e.g., gyroscopes, integrated circuits, displays, keypads, enclosures, etc.) have been acquired for about 20 units. An intervention study to assess the effectiveness of the AVTD's in reducing the veering of blind pedestrians is scheduled for Year 5, following laboratory testing of the fully assembled units.

VANDERBILT UNIVERSITY

During the past year the Vanderbilt team has developed evidence-based recommendations for optimal placement of accessible pedestrian signals to inform pedestrians which crosswalk has the walk signal active. Software and hardware development was completed for a "virtual reality" method of presenting sounds from two fixed loudspeakers, so as to create the impression that a sound comes from any desired direction in the horizontal plane. The first validation experiment of this technology has been completed. Ongoing work is evaluating a four-loudspeaker system for virtual sounds, which may enhance the realism of sounds presented from behind a listener. Also, the virtual system is being used in an experiment on perception of moving sound sources, with a focus on curvilinear motion paths that occur in the roadway environment.

MARYLAND SCHOOL FOR THE BLIND
During year 4, MSB/Johns Hopkins researchers completed a study of driver yielding behavior in response to pedestrians who are blind at roundabout intersections.
Data collection has begun on two additional studies. The first will evaluate the effect of exposure time on gap judgments at a roundabout intersection. Participants who have normal vision or who are legally blind due to glaucoma or retinitis pigmentosa will receive varying amounts of visual preview of traffic conditions as they complete a gap judgment task. This will allow examination of the relationship between visual status and the accuracy and speed of crossing judgments. In the second study, researchers will evaluate the relationship between visual impairment, visual processing speed, and risk tolerance at roundabouts.

BOSTON COLLEGE
In Year 4, accessible pedestrian signals were installed at the two complex signalized intersections in each of four cities where pretest street crossing data were collected (Portland, OR, San Diego, CA, Cambridge, MA, Charlotte, NC). Pre and posttest data have been collected from sixteen blind participants, who each made approximately four crossings at two intersections in each city. In year five, the signals will be modified in these cities based on the results of Year 4 testing, and data will be collected again to determine if the modifications yields benefits to pedestrians crossing without visual cues. The pre-installation testing yielded the first documentation of how blind pedestrians actually cross at complex signalized intersections. These data are critical for our understanding of travel behavior in these environments.

UNIVERSITY OF NORTH CAROLINA HIGHWAY SAFETY RESEARCH CENTER AND NORTH CAROLINA STATE UNIVERSITY INSTITUTE ON TRANSPORTATION RESEARCH AND EDUCATION
UNC's Highway Safety Research Center, along with North Carolina State's Institute for Transportation Research and Education, have explored the use of traffic engineering modeling tools to better understand pedestrian and vehicle operations at roundabout intersections, and to apply modeling strategies to the issues of access by blind pedestrians. Researchers integrated blind and sighted pedestrian behavioral data collected by other teams into the VISSIM traffic engineering model. ITRE staff has continued work on the development of the ITRE-mv, a video image processing system. The objective of this system is to automate the collection of traffic data from video-tapes to be obtained in future studies

The partnership staff has submitted or published 12 papers and made 25 conference papers, and it has significantly influenced the development of federal research programs and regulatory activities in regard to intersection access for persons who are blind or visually impaired.

ISSUES
None identified

PI: Duncan Maitland, Ph.D.
Medical Physics and BioPhysics Division
Lawrence Livermore National Laboratory
7000 East Avenue, L-211
Livermore, CA 94550
Tel: 925-423-6697
Email: dmaitland@llnl.gov

PROJECT TITLE: Shape Memory Polymer Devices for Treating Stroke

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

PARTNERS' NAMES AND AFFILIATIONS:
Jon Hartman, M.D. (UC Davis, Radiology Dept.)
Judy Van de Water, Ph.D. (UC Davis, Rheumatology/Allergy Dept.)
Ted Wun, M.D. (UC Davis, Hematology Dept.)
Scott Simon, Ph.D. (UC Davis, BME Dept.)
Robert Gunther, Ph.D. (UC Davis Dept. of Surgery)
Bill Ferrier, D.V.M. (UC Davis)
Thomas Wilson, Ph.D. (LLNL)
Jane Bearinger, Ph.D. (LLNL)
Ward Small, Ph.D. (LLNL)

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 second year research goals set forth in the proposal. . The key aims of the second year are animal trials with the second-generation devices. The first generation stents have been fabricated and preliminary animal trials accomplished. A working neurovascular stent delivery system is expected by the end of the third year. The development of both the clot extraction and polymer stent devices are progressing well. All of the project components including device engineering, materials development and characterization, biocompatibility studies and interventional studies are under way. . The ischemic stroke devices have been integrated into standard guide wire (coil) and catheter (basket) devices and there is a strong possibility that they could lead to commercial devices after the animal trials. The in vitro biocompatibility studies show that SMP is essentially equivalent to Teflon for cytokine and platelet activation. The first stent implant studies show no inflammation in rabbit carotid arteries. Functional animal studies of the acute ischemic stroke device are underway. Beyond the original scope of the proposal, we had, and took, the opportunity to undertake preliminary studies in three distinct areas: shape memory foams, synthesize new shape memory polymer materials (two different materials, acrylic and a different urethane from the Mitsubishi materials), and magnetic field heating/actuation of the materials. These spin-off studies have concluded are being written up as publications. We have also received seed funding for both the foam (NIH SBIR with Sierra Interventions, Melodie Metzger is the PI, Duncan Maitland is the LLNL co-I) and new materials (LLNL internal peer review award, Tom Wilson is the PI).

ISSUES

There are no significant issues on the project.

PI: Mark Roger, MD, PhD
Massachusetts Institute of Technology
Division of Health Sciences and Technology
Room E25-505
Cambridge, MA 02139
T: 617-253-7818
F: 617-258-7859
rgmark@mit.edu

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

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

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

This research effort will collect and annotate an extensive and comprehensive new database from ICU patients to support research in intelligent patient monitoring. The database will contain continuous waveforms, multi-parameter trends, progress notes, medication records and laboratory data from 500 patients throughout their ICU stay. The de-identified database will be made freely available to the research community when completed. Innovative and sophisticated algorithms and clinician interfaces will be developed to assist in the annotation effort and to create a prototype advanced monitoring system. Evaluation of the new tools and displays will begin in the laboratory utilizing the new database. Later,

industry-constructed monitoring system prototypes will be deployed and evaluated in clinical settings at Beth Israel Deaconess Medical Center.

STATUS OF RESEARCH AND PARTNERSHIP

During the initial eight months of the project significant progress has been made in three major focus areas:

Data – We have collected records from 3,000 patients from medical, cardiac, and surgical ICUs. The data occupies almost one terabyte of storage (the MIMIC II Database). We have designed and completed construction of a sophisticated annotation system that supports expert clinicians to identify significant physiologic events in the records (e.g. pulmonary edema, hemorrhage, cardiogenic shock), and to link to relevant supporting evidence in nursing notes, labs, therapies, trends or waveforms. Annotations and supporting evidence is semi-automatically coded using the SNOMED-CT vocabulary. We have made substantial progress in developing automated de-identification software.

Models - Much of our effort is aimed at implementing model-based approaches to evaluation, filtering and integration of ICU data, tracking of patient state, reasoning about patient condition, alarm generation, and so on. The static and dynamic models involved are embodied in computer simulations. We have been revisiting our existing implementations of cardiovascular simulations in C and Matlab, and also setting up parallel implementations in the circuit simulation program H-Spice (taking advantage of the fact that our models are largely specified in terms of circuit analogs). Given that our simulations are likely to run over hundreds or even thousands of cardiac cycles, there is a big potential payoff in using averaged models rather than pulsatile ones. The pulsatile models generate the detailed intra-cycle waveforms, whereas the averaged ones simply track the dynamics of the cycle-averaged variables, and can therefore run much faster.

Reasoning - Work focused on the problems of extracting medically relevant meaning from unstructured English text. We concentrated on two different kinds of sources of text because we believe that they pose significantly different challenges. These are: (1) nursing notes, which are highly telegraphic, information-packed and idiosyncratic notes written during clinical care by ICU nurses, and (2) more formal admitting, progress and discharge notes, which tend to be written with less condensation. These cover the range of tasks that will naturally arise in extracting meaning from unstructured text in the ICU domain.

The partnership:

The partnership is functioning smoothly, and each partner is critical to the feasibility and success of the project. The Beth Israel Deaconess Medical Center has facilitated data gathering from all ICU beds on the west campus. We will appoint a BIDMC physician as a post-doc on the project for the next two years to participate in data acquisition, annotation, and analysis. MIT is the site for data archiving and analysis (Prof. Mark), modeling (Prof. Verghese), and reasoning/expert systems (Prof Szolovits). The Philips Company supports data collection by contributing critical hardware and custom software without which we could have no access to the data, and their engineers in Andover and in Briarcliff Manor, NY participate with us as research colleagues.

ISSUES:

We are exploring a new collaboration with the University of Massachusetts Medical Center to collect data from their ICUs, with particular emphasis on the Pediatric ICU. This will enlarge the scope of the MIMIC database to include children, and will add another highly qualified collaborator, Dr. Joseph Frassica, who directs the PICU.

PI: Michael A. Matthews
University of South Carolina
Department of Chemical Engineering
Swearingen Engineering Center
Columbia, SC 29208
T: (803) 777-0556
F: (803) 777-0973
matthews@engr.sc.edu
www.che.sc.edu.

PROJECT TITLE: Processing of Materials for Improved Biocompatibility

PARTNERS' NAMES AND AFFILIATIONS:
Yuehuei An, MD (Medical University of South Carolina), Martine LaBerge, PhD and Michael Drews, PhD (Clemson University), John Keller, PhD (University of Iowa), Lalit Chordia, PhD (Thar Technologies)

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

ABSTRACT
The overall goal for this research partnership is to obtain fundamental understanding of a novel, low temperature process for sterilizing and cleaning of 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 cleaning and sterilization based on liquid or highly compressed carbon dioxide (CO_2), and 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. The research will support the development of the next generation of biomaterials (e.g. for tissue engineering) that are not compatible with current methods of sterilization and cleaning. This process has potential to eliminate material damage associated with irradiation. It will also eliminate the need for toxic sterilizing agents such as ethylene oxide, which tends to reside in polymeric materials.

This Bioengineering Research Partnership project will address seven fundamental questions, namely: (1) What conditions produce complete sterilization? (2) At what conditions does CO_2 remove soluble contaminants from biomaterials? (3) What are the mechanisms of CO_2 sterilization? (4) At what conditions are particulates (i.e., metal or plastic residues of machining), microorganisms, endotoxins, and other cellular debris, removed by dense phase carbon dioxide-based fluids? (5) Can biomaterials be cleaned and sterilized effectively without damage to the surface or material properties? (6) How do specific material limitations affect the development of a CO_2-based process? (7) What are the underlying physical, chemical, and biochemical mechanisms of cleaning and sterilization?

STATUS OF RESEARCH AND PARTNERSHIP
Three papers have been submitted to peer-reviewed journals and two more are in preparation. Regarding the conditions and mechanisms of sterilization, we have narrowed a region of temperature, pressure, and composition that gives substantial deactivation of all three spores at mild temperatures. Significant reduction in *B. pumilis* and *B. Atrophaeus* spore viability has been achieved between 40 and 60 ^oC using carbon dioxide with low (ppm) levels of aqueous H_2O_2. Spore strips (10^6 cfu/strip) have been treated using CO_2 in conjunction with additives, including water, ethanol, isopropanol, and hydrogen peroxide, at temperatures in the range of

40 – 80°C, and pressures of 1500 or 4000 psi. The methodology includes appropriate controls. Five microliters of 30% hydrogen peroxide in a 10-ml chamber volume enhanced spore deactivation to 4.45-log at 40°C. The deactivation effect increases to 6.28 log reduction (i.e. 100% spore kill) using five microliter of 30% hydrogen peroxide with carbon dioxide at 60°C, 4000 psi, for four hours. Complete deactivation of *G. Stearothermophilus* spores occurred in two hours at 40 ˚C at a somewhat higher pressure, 7,200 psi. Published data has also shown that significantly higher concentrations (30%) of aqueous H_2O_2 are generally needed for deactivation of *G. stearothermophilus* These results suggest a synergistic mechanism between the CO_2 and the H_2O_2 , resulting in more effective sterilization. Our mechanistic studies will shed more insight on this synergy.

Regarding the mechanisms of sterilization, we have examined changes in morphology of spores using scanning electron microscopy. A novel staining protocol using ruthenium red (a carbohydrate stain) has revealed possible structural changes in the exosporium due to CO_2 treatment. Using JOEL model 200CX TEM for visualization, *B. atrophaeus* spores treated with high pressure CO_2 and 5 μl of 30% H_2O_2 displayed a fibrous exosporium that showed signs of physical disruption. If high pressure CO_2 does indeed disrupt the exosporium, this could allow H_2O_2 to diffuse into the spore to achieve deactivation. Molecular analysis of the matrix is needed to confirm this hypothesis, and these studies are planned for Year 3.

The effect of CO_2 processing on mechanical properties of clinically relevant polymers is also an area of importance. We have studied the property changes caused by two common U.S. sterilization methods (ethylene oxide gas (EtO) and γ-radiation) and compared these to the changes caused by CO_2 sterilization. The polymers utilized were medical grade ultra high molecular weight polyethylene (UHMWPE) (from Poly Hi Solidur, Inc, Fort Wayne, IN), silicone (.35 mm sheets from Mc-Master Carr, Atlanta, GA) and polyurethane (Tecoflex® SG-93A obtained from Thermedics Polymer Products, Woburn, MA). The properties of CO_2 sterilized materials were compared to samples sterilized by EtO and γ-sterilization as well as an untreated control group. For all of the polymers studied, exposure to the CO_2 sterilization cycle caused minimal effect on the mechanical properties such as elastic modulus, ultimate tensile stress, storage and loss modulus. Only with the Tecoflex® polyurethane was a change in appearance as well as surface roughness observed. During sterilization the clear films became opaque, suggesting the formation of bubbles during the depressurization step following sterilization with SC-CO_2.

Our planned work is as follows: The elucidation of the mechanisms of sterilization will continue using TEM as well as SEM. In addition, a fluorescent live/dead assay (Baclight™) will be used to assess whether the inner membranes of the spores are compromised during SC-CO_2 treatment (as is the case with wet heat killed spores) or whether the inner membranes are unaffected (as is typically the case with H_2O_2 killed spores). Other ongoing work includes the use of a pH dependent fluorescent assay (BCECF) in order to determine whether or not the exposure to SC-CO_2 significantly lowers the internal pH of the spores, as has been proposed by some researchers. Work is also in progress on the development of both colorimetric and fluorescent assays that will allow the determination of the extent of dipicolinic acid (DPA) leakage from inside the spores, which would be another indication of internal membrane rupture. We also plan to look for changes in the spore coat carbohydrate content. Work is in progress on comparing the effect of CO_2 to that of EtO and γ-sterilization on the material properties of a bio-absorbable polymer, polylactic acid (PLA) films. Work continues on finding the appropriate additives to facilitate bacterial removal. In addition, experiments in progress are designed to study the effect of SC-CO_2 sterilization on the wear behavior of UHMWPE. A major task in year 3 is the in vivo biocompatibility testing in a rat subcutaneous implantation model. The in vivo experiment will be conducted as described in the original proposal.

ISSUES

Industrial interest in this project remains high, and the BRP team is investigating intellectual property issues that may arise when commercial development opportunities develop. We have also placed two grant-supported students on industrial internships. Collaborations with basic medical sciences are increasing, particularly in the areas of ultrastructural and biochemical characterization. As we approach the renewal of this grant, it is highly likely that new partners will come from medical sciences as as well as one or more additional companies.

PI: Andrew Maudsley, Ph.D.
MR Center (R308)
University of Miami School of Medicine
1115 N.W. 14th St.
Miami, FL 33136
Tel: 305-243-8080
AMaudsley@med.miami.edu
http://midas.med.miami.edu/

PROJECT TITLE: Partnership for MR spectroscopic Imaging Data Processing

PARTNERS' NAMES AND AFILIATIONS:

Andrew A. Maudsley, Ph.D.	Department of Radiology, University of Miami
Jeffry R. Alger, Ph.D.	Department of Radiology, University of California Los Angeles
Lawrence O. Hall, Ph.D.	Computer Science and Engineering, University of South Florida,
Norbert Schuff, Ph.D.	Northern California Institute for Research and Education and Department of Radiology, University of California San Francisco
Colin Studholme, Ph.D.	Northern California Institute for Research and Education and Department of Radiology, University of California San Francisco

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 requirements for data processing and analysis. 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 develop an integrated set of processing tools that satisfy these requirements, thereby simplifying implementation of MRSI for routine diagnostic imaging studies of the brain and increasing the potential information content.
This effort combines development of MRSI and MRI data processing software under 5 projects located at 4 institutions. Once developed, this will be followed by data acquisition at multiple world-wide collaborative sites. Software will be developed for automated MRSI processing, tissue segmentation, brain region mapping, statistical analysis, and clinical presentation. Results from MRSI and MRI studies will be converted to standardized intensity units and transformed into normalized spatial coordinates, enabling the data to be pooled to form a database of MR-measured human metabolite values. This information will then be used to enhance statistical analysis of individual MRSI studies and map metabolite distributions in normal human brain. The resultant technical developments will be shared among several partners at collaborating medical research centers in the U.S.A., Europe, and Japan, where the package will be evaluated for diagnostic neuroimaging applications, with an emphasis on 1H MRSI of cancer, epilepsy, and neurodegenerative disease.

STATUS OF RESEARCH AND PARTNERSHIP

In this second year, additional effort has been placed in developing the XML-based information management system that will organize the multiple MRI and MRSI data sets obtained, as well as the results of the data processing and analysis. This includes a data import module that supports standard DICOM MRI formats together with MRS data import support, which is currently implemented differently by each instrument manufacturer. Several sample data sets have been obtained from normal human brain for the purposes of testing the developed software, though acquisition of additional studies has been delayed until the database management system is fully in place. With the increased availability of 3T MRI

systems, and their expected advantages for the MRSI studies planned in this project, it has been decided that the project should support data acquisitions in normal subjects at both the originally-planned 1.5 T, and at 3.0T. Based on preliminary data obtained from 3 T MRI systems, it appears that this will have several new considerations, including: different settings used for tissue segmentation; image distortions and alignment between multiple MRI data types; and acquisition parameters for MRSI. These considerations will add additional effort to the project.
Progress from individual projects includes development of metabolite calibration methods (Project 1); verification of tissue segmentation algorithms and accuracy (Project 2); definition of the brain atlas and reference MRI (Project 3); prototyping of statistical image analysis programs (Project 4); and progress with programming of a Java-base MRI and MRSI image viewer for the clinician (Project 5).
The Web server established in year one to has proven to be effective for managing software version control (CVS) and for distribution of data. A set of library functions has been written in Java that supports the XML database format, and distributed via the CVS system. Meanwhile, each project has proceeded with developing the functionality needed for each section, but this will now need to be modified to use the newly-defined XML data management system.
Both a PIs meeting and individual programmer meetings held during this last year have proven to be very useful. In addition, conference calls have been used extensively to coordinate development efforts.

ISSUES
The requirement for a comprehensive data management system was not anticipated in the initial proposal, and this development effort has required additional resources. Although the partner sites have been able to continue development as planned, the lack of the common data format has delayed integration of the different software components.

PI: Larry V. McIntire, Ph.D.
Wallace H. Coulter Professor & Chair
The Wallace H. Coulter Department of Biomedical Engineering
at Georgia Tech and Emory University
313 Ferst Drive
Atlanta, GA 30332-0535
larry.mcintire@bme.gatech.edu

PROJECT TITLE: Leukocyte Trafficking From Flowing Blood Tissue

PARTNERS' NAMES AND AFFILIATIONS:

Suzanne Eskin (Georgia Institute of Technology), Jennifer West (Rice University), Thomas Chou (Rice University), Wayne Smith (Baylor College of Medicine), Michele Mariscalco (Baylor College of Medicine), Alan Burns (Baylor College of Medicine), Samina Kanwar (Baylor College of Medicine)

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

ABSTRACT

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

STATUS OF RESEARCH AND PARTNERSHIP

Our BRP employs tissue engineering technologies to evaluate neutrophil interactions with the extracellular matrix (ECM)-mimetic peptides in two and three dimensional systems. We have used a polyethylene glycol (PEG) diacrylate derivative to form a hydrogel that provides a biologically inert surface. Covalently attaching bioactive moieties into the hydrogel has made it bioactive. The goal is to define the mechanisms by which these moieties influence the interactions of neutrophils with this

bioactive hydrogel, and thus understand the likely effects of similar ligands in the ECM. These findings will then be tested in vivo employing a murine model. The current experiments analyze the interactions of isolated human neutrophils with PEG hydrogels modified with Arg-Gly-Asp-Ser (RGDS), known ligand for some β1 and β3 integrins, and Thr-Mer-Lys-Ile-Ile-Pro-Phe-Asn-Arg-Leu-Thr-Ile-Gly-Gly (TMKIIPFNRLTIGG), ligand for Mac-1, a β2 integrin. Our results demonstrate that neutrophils, independent of chemotactic stimulation, show little ability to adhere to unmodified PEG hydrogels. However, cell adhesion and spreading are robust on peptide-modified hydrogels. Incorporating distinct bioactive peptides, either alone or in combination, has enabled recognition of differential functions of αvβ3, β1 and β2 integrins on neutrophil adhesion and spreading. Combined interactions result in activity that differs markedly from that seen with either integrin independently engaged. This model allows investigation of specific ligand-induced leukocyte functions and the development of engineered matrices with defined bioactive properties. Studies on motility (quantitated with video microscopy) have just begun. We are in the first year of our partnership and progress has been quite good. We meet monthly via videoconference for 2 hours. This allows detailed discussion of data obtained (2 way video including use of powerpoint presentations), analysis of potential problems and the planning of future experiments. I travel to Houston every two months to directly interact with my Co-PI's and research staff at Baylor College of Medicine and Rice University.

ISSUES:

Challenges include maintaining good information flow – as each laboratory has areas of special expertise that all in the partnership need to be able to use to accomplish our multidisciplinary goals. We are optimistic that progress will continue to be rapid.

PI: Timothy McKnight, M.S.
Oak Ridge National Laboratory
Jointly appointed with the Molecular Scale Engineering and Nanoscale Technologies Research Group of the Engineering Science and Technology Division and the Biological Materials Research Group of the Condensed Matter Sciences Division
1 Bethel Valley Road
Oak Ridge, TN 37831-6004
T: 865-574-5681 F: 865-574-1249 email:mcknightte@ornl.gov www.ornl.gov/ment

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

PARTNERS' NAMES AND AFFILIATIONS:
Michael Guillorn (ORNL/Cornell), Vladimir Merkulov (ORNL) , Anatoli Melechko (ORNL/University of Tennessee), Guy Griffin (ORNL), Michael Simpson (ORNL/University of Tennessee), Stephen Jacobson (Indiana University), Chris Culbertson (University of Kansas), Phillip Rack (Dept. Materials Science and Engineering, University of Tennessee), Seung-Ik Jun (Dept. Materials Science and Engineering, University of Tennessee)

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. They may be deterministically synthesized on a variety of substrates (silicon, quartz, glass), with a high level of control over many parameters including length, tip diameter, aspect ratio, physical location on the substrate, and surface chemistry. In this effort, nanofiber probing arrays are being fabricated into devices that feature individually-addressable, nanofiber based electrochemical electrodes where only the extreme nanoscale tip of the fiber is electrochemically active. The nanofiber serves both to elevate the electroanalytical measurement volume above the planar substrate (i.e. within and around cells as opposed to in-between the substrate and cellular matrix) and to electrically bridge between the nanoscale dimensions of the fiber tip and the microscale dimensions of the electrical interconnects of the substrate. Additionally, in this research effort, nanofiber-device fabrication approaches are structured around incorporation of microfluidic cell and analyte handling strategies, thereby providing architectures that will enable future high-throughput screening applications, such as clinical diagnostics of cell and tissue specimens and pharmaceutical exploration and discovery.
Research tasks are focused around several aims: fabrication of robust, nanofiber-based probing architectures; electroanalytical characterization of nanofiber-based electrodes against benchmark analytes as well as biologically-relevant species; investigation of cell/fiber interfacing schemes; and, ultimately, measurement of electrochemically-active species in and around cellular matrices. Reactive oxygen species (ROS; specifically hydrogen peroxide and superoxide anion) were selected as the target bioanalytes due to their critical role in virtually every aspect of cell function.

STATUS OF RESEARCH AND PARTNERSHIP:
In the second year of this effort, we have optimized the fabrication of nanofiber-based electrochemical probe architectures (aim 1), fully characterized the electrochemical performance of these evolving architectures (aim 2), investigated functional enzyme immobilization strategies for ROS, specifically hydrogen peroxide (aim 3), and are continuing to develop methods for integrating functional nanofiber

elements with the intracellular and extracellular domains of viable cell matrixes including those that make use of microfluidic and elastomeric actuator manipulation of single cells (aim 4). In addition, we have leveraged supplemental grant funds to begin to realize architectures that provide higher levels of parallelism (higher numbers of addressable probing elements) in nanofiber-based measurements – both using optical addressing strategies and active arrays to activate individual probing elements (1 yr funding supplement).

Nanofiber probe arrays are now routinely fabricated to feature 40 individually-addressable nanofiber elements within a 30 micron-wide fluidic channel. The current design layout provides electrode spacing at down to 2 micron interfiber spacing, thereby enabling the potential of 4 probing elements around or within even the smallest of mammalian cells. Methods to promote attachment and proliferation of a variety of cell types, including Chinese hamster ovary (CHO), rat thoracic aorta (A10), and quail neuroretina (QNR), have been developed and implemented on fully packaged, functional devices (5 mm square, with 40 interconnect to external instrumentation).

We have advanced our analytical study of the electrochemistry of nanofiber devices to include characterization of these individually addressable architectures (McKnight TE, et al. Microarrays of Vertically-Aligned Carbon Nanofiber Electrodes in an Open Fluidic Channel, J. Phys. Chem. B 108(22): 7115-7125. 2004). We have demonstrated control over the physical dimensions of the electrochemical surface area of probing electrodes to provide sub-100 nm active probe tips. These active probing elements are microfabricated in fluidic manifolds that provide structural integration of probes with cellular matrices, in addition to integrated fluidic handling strategies. While this effort focuses on integration of these structures with live cells and tissue, we anticipate the application of these fluidically-integrated electrode structures will also be of interest to the lab-on-a-chip and scanning electrochemical microscopy communities for in-channel probing of chemical separations, etc. Of particular significance is our demonstration of generator-collector feedback measurements between adjacent nanoscale probes at micron separation distances. Application of this technique has considerable potential for multimodal measurements within fluidic channels and for investigating the fate of electrogenerated species in and around cells.

We have continued to develop functionalization strategies for providing probing specificity to desired analytes. We have demonstrating the direct covalent tethering of functional enzymes and amino acids to individual probing elements (McKnight TE, et al. Tracking Gene Expression after DNA Delivery Using Spatially Indexed Nanofiber Arrays, NanoLetters, 2004 ASAP), as well as the capture of active enzymes in electropolymerized electronically conducting coatings.

ISSUES:
None.

PI: David Meaney
120 Hayden Hall
Department of Bioengineering
University of Pennsylvania
3320 Smith Walk
Philadelphia, PA 19104-6392
T: 215-573-3155
dmeaney@seas.upenn.edu

PROJECT TITLE: Molecular expression of force transmission in the central nervous system

PARTNERS' NAMES AND AFFILIATIONS: Departments of Pharmacology, Neurosurgery, Genetics, and Bioengineering, University of Pennsylvania; Department of Neuropathology, University of Glasgow; Novartis, Inc.

GRANTING INSTITUTE: NICHD

ABSTRACT
The partnership is designed around three central areas: cellular mechanics, molecular measurement technologies, and cellular informatics. These areas are integrated and applied to the study of traumatic injury to the CNS. Our main objectives are to develop the appropriate technological infrastructure to study, at the single cell level, the heterogeneity of events that occur within a traumatically injured neuron. We propose that a unique set of molecular events at the transcriptional/translational level, mediated through immediate biochemical changes in the cell, will direct the fate of individual neurons in the traumatically injured brain. The vast extent of information available for identifying these unique characteristics is significant, and will require an informatics based approach to decipher the important events from the less significant changes in the cell. In addition, we need to expand and develop moderate throughout technologies to accommodate more ready screening of compounds to test for therapies in the injured brain. We focus on the changes that occur with apoptotic and necrotic cell death in the cortex, using a combination of in vivo and in vitro models in identifying the appropriate targets for intervening to repair neurons in the brain after injury.

STATUS OF THE RESEARCH PARTNERSHIP
In the area of cellular mechanics, we have tracked the motion of cellular structures in organotypic culture models, and have used these to develop mathematical models for the cellular motions that occur in different brain regions. From these analyses, we estimate the deformation field experienced by cells in regions that are frequently injured in traumatic brain injury. In parallel, we have completed studies to describe the functional response of these cells is dissociated and organotypic culture, and have found that (a) specific receptor populations are mechanosensitive, and the receptor subunit composition dictates the mechanosensitivity, (b) the stretch threshold for cell death is different among separate regions in the hippocampus, and (c) changes in both synaptic and extrasynaptic receptors contribute to neuronal death. These data provide important complementary information for developing a on specific gene expression profiles generated within traumatically neurons, as well
as developing potential therapeutics.
For molecular measurement technologies, work in the past year has focused in two areas. First, we have now completed work to isolate mRNA from individual neurons within the traumatically injured brain. We showed that overall expression of mRNAs increased with activation of caspase 3 and decreased to below uninjured levels with TUNEL reactivity. Cell type specificity of the apoptotic response was observed with both regionally distinct expression of mRNAs and differences in those mRNAs that were maximally regulated. Immunohistochemical analysis for two of the most highly differentially expressed

genes (prion and Sos2) demonstrated a correlation between the observed differential gene expression after traumatic brain injury and corresponding protein translation.

Based on this data, we felt it was important to identify which of these genes are preferentially targeted and transported within the neuron. We chose to examine KIF5A, a kinesin isoform that is known to influence dendritic transport. KIF5A immunoprecipitation yielded material that was then amplified using aRNA amplification, and the resulting amplified material was put on microarrays for identification of RNA species present in the sample. From this work, approximately 65 candidates were present on the array with a ratio of sample:control in excess of 10. These candidates are being screened for direct KIF5A binding using filter binding assays with recombinant kinesin cloned in the past year. Gene profiles of isolated dendrites from transfected, KIF5A-depleted cells will be compared to profiles of normal control dendrites following mechanical injury, attempting to show the next level of gene regulation after trauma – the differential transport of mRNA throughout the cell. Additional studies are planned for the identification (through mass spectrometry and protein microsequencing) of other proteins that are associated with the kinesin motor complex and present in the immunoprecipitate.

ISSUES

Based on the results from some of the mechanotransduction studies, we have secured an agreement with a corporate partner to begin screening compounds in an experimental model of TBI. We will report on the results from this study at next year's meeting.

PI: Srboljub Mijailovich, Ph.D.
Harvard School of Public Health
Environmental Health
665 Huntington Ave
Boston, MA 02115
T: 617-432-4814
F: 617-432-4710
smijailo@hsph.harvard.edu
www.hsph.harvard.edu

PARTNERS' NAMES AND AFFILIATIONS:
Michael A. Geeves (University of Kent, UK), Richard L. Moss (University of Wisconsin), David A. Smith (Monash University, Australia), John Sleep (King's College London, UK), Kenneth, B. Campbell (Washington State University)

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

PROJECT TITLE: Quantitative Bioengineering Analysis of Muscle Mechanics and Metabolism

ABSTRACT
Using the methods of engineering analysis, we will develop 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 addresse 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) transitional rate constants in 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

Overall, our research is progressing well. Although we have been funded for only ten months, we already have one paper published and another is currently being reviewed. We have also presented several abstracts at national and international conferences in 2003 and 2004. Specific Aim 1 is ahead of schedule: (1) we have developed a modular structure of a computational platform that combines probabilistic actomyosin binding kinetics with **finite element analysis** (which takes in account the thick and thin filaments); (2) we are now in process of developing the first version of a three dimensional model of sarcomeric lattice with extensible filaments, using an updated biochemical cross-bridge cycle and models of stochastic myosin binding; and (3) Dr. Geeves is investigating the correlation of biochemistry and mechanical data on the cross bridge cycle. Specific Aim 2 is in the initial phase: (1) we are currently preparing foundations for the thin filament regulation models in solution and 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 have developed an *in vitro* system which involves applying a (virtual) load on a uniaxially stretched smooth muscle strip to mimic the physiological (dynamic) load experienced by intact smooth muscle cells in airways and blood vessels.

The BRP partners met for a two-day meeting (Boston, August 2003) and one-day meeting (Baltimore, February 2004). These meetings have been intense, mutually beneficial and highly productive. At the first meeting we established an initial plan and priorities for the first year, and at the second meeting we reviewed the progress within first six months of the project. In our discussions we defined the milestones to be achieved in the coming year at each of the project sites. We have turned our attention to Aims 1 and 2 in order to make substantial progress that will lead to several publications within the Year 2 of the project. We continue to develop new software, hardware, and experimental protocols.

ISSUES

None.

PI: Ronald N. Miles, Ph.D.
State University of New York at Binghamton
Department of Mechanical Engineering
P.O. Box 6000, Binghamton, NY 13902.
T: (607) 777-4038
F: (607) 777-4620
miles@binghamton.edu
http://www.me.binghamton.edu/

PARTNERS' NAMES AND AFFILIATIONS:
Dorel Homentcovschi (State University of New York at Binghamton), Ron R. Hoy (Cornell University), Levent F. Degertekin (Georgia Institute of Technology), Peter Loeppert (Knowles Electronics), Steven Thompson (Knowles Electronics), Douglas Jones (University of Illinois at Urbana-Champaign),

GRANTING NIH INSTITUTE/CENTER: National Institute on Deafness and Other Communications Disorders (NIHDCD)

PROJECT TITLE: Sensing and Processing for Directional Hearing Aids

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

The research efforts for year one have focused on the following three major tasks: developing an optimized design for the microphone diaphragms; developing a fabrication process for incorporating optical sensing; developing signal processing algorithms for enhancing noise reduction and experimentally verifying these algorithms with currently available directional microphones. With respect to these top-level tasks, the highlights of sub-tasks have been completed: experimental characterization has been completed on the first set of microphones diaphragms; analytical models for the performance of various microphone designs have been completed, current fabrication efforts include the construction of 12 photolithographic masks to be used for various layers in our wafer processing; development of a fabrication process for optical sensing; the construction of a BTE-mounted test array of two omnidirectional microphones; the design of an optimal fixed beamformer algorithm that maximizes the directivity index by exploiting HRTF and multi-element microphones; an analysis of necessary characteristics for the optical grating design; the development of a bench-top optical detection scheme to enable a macro-scale examination of optical sensing designs; the resolution of initial package design issues; the construction of the first fully integrated optical microphones using a previously designed device (fabricated at Sandia) as the microphone diaphragm; and the design of an integrated circuit including VCSEL driver, photodiodes and transimpedance amplifiers.

ISSUES

Approval and funding of this project began approximately one month into the fiscal year (Sep 26, 2003) which delayed the non-disclosure and subcontract agreements process and the recruitment of personnel. Personnel for SUNY and GT were hired by mid-January 2004, approximately 3 months behind schedule. UIUC did not have sufficient time within their academic cycle to hire needed personnel, which resulted in one of their 'non-critical path' tasks, Scene Analysis in the task #10.4 family, being pushed into year 2. The project schedule has been adjusted to accommodate these delays and maintain the project timeline. Also, due to an initial budget reduction, it was necessary to revise the team structure and reallocate fabrication tasks. A revised statement of work was completed and approved.

PI: Wayne Mitzner, Ph.D.
Johns Hopkins Bloomberg School of Public Health
T: 410 614 5446
F: 410 955 0299
wmitzner@jhsph.edu
www.ephysiology.org

PROJECT TITLE: New Approach for the Treatment of Asthma

PARTNERS:
Asthmatx, Inc.
1340 Space Park Way
Mountain View, CA 94043
T: 650-810-1100
F: 650-810-1101

GRANTING INSTITUTE: NHLBI

ABSTRACT
This proposal will develop and evaluate an innovative and potential clinical treatment for asthma. Although there are a multitude of different possible triggers, an acute asthmatic attack is always characterized by contraction of the smooth muscle in the airway wall. Despite this common end point, most of the clinical asthma research and therapies in recent years have focused on understanding the immunologic factors that often lead to asthmatic attacks. The present proposal describes research and development that focuses on a treatment of smooth muscle that will thus be effective in asthmatic attacks regardless of the initial trigger. It involves the design, construction, and application of a biomedical device that can prevent or minimize the ability of the smooth muscle in the airways to constrict. The project involves a close working partnership between the physiologic laboratories and expertise at the Johns Hopkins University and Asthmatx, Inc, a small biomedical engineering company in Mountain View . Asthmatx provides the mechanical and bioengineering expertise required for product development. The overall hypothesis governing this proposal is that, the treatment of airway smooth muscle with this innovative system will minimize obstruction caused by smooth muscle contraction, regardless of its origin. In this proposal, six specific aims will be directed toward addressing this hypothesis. Two aims are concerned with determining the optimal parameters and design criteria for maximal effectiveness, and four aims are concerned with assessing the safety and potential side effects of this treatment. The information obtained from these functional studies will be essential, not only in the ongoing engineering and development of an optimal device, but also to help set guidelines for the use of this device in future clinical trials. The studies proposed in this BRP will thus allow optimization of a biomedical device that has the potential to effectively cure all forms of human asthma.

STATUS OF RESEARCH AND PARTNERSHIP
Results in the third year of this partnership have focused on experimental work using high resolution CT imaging to examine the responsiveness of airways treated with the current prototype device that delivers energy to the airway wall through a bronchoscope. Work was initiated and partially completed on the first 2 specific aims in the original proposal. The first aim is concerned with device modifications and optimization. To this end, Asthmatx has developed a custom R&D software platform for the RF Generator, and a device with more user control for animal experimentation. This device allows programmable adjustment of all key energy delivery parameters, including temperature control algorithm parameters. Previous software versions had fixed control parameters.

Work at Asthmatx has focused on further development of the computer model that simulates the generation and conduction of heat in the lungs during the application of RF energy to the airway wall. The model uses software from the Computational Fluid Dynamics Research Corporation which allows the transient solution of coupled partial differential equations. The modeling work to date has demonstrated the effects of variations in tissue properties of the airway wall with respect to the surrounding parenchyma. This model has also clearly shown that the differences in electrical and thermal conductivity between the airway wall and the parenchyma significantly affect the resulting transient temperature distribution in the airway wall and the parenchyma. This work was recently presented at the ATS meeting in Orlando. As the temperature time history is a key factor in determining the effect of treatment, this provides valuable information in our attempts to target ASM more selectively. We have also found qualitative agreement between the modeling results and histology observations. Further work will include modeling the effects of anatomical heterogeneities, determination of tissue properties, and *in vivo* confirmatory experiments.

Experimental work at Johns Hopkins in the past year was concerned with two experiments. We have completed one protocol showing that airways treated with the device do not contract to as small a size with any dose of inhaled agonist (methacholine). In another new study, which is nearing completion, we have been examining the effectiveness of treatment against the ability of airways to close completely. We had previously developed a system to challenge airways sufficiently with agonist to make them close, and we are using this technology to study the effectiveness of Alair treatment in preventing this.

ISSUES: No substantive issues at this time. We are anticipating submitting a competitive renewal next summer.

PI: Carol Muehleman , Ph.D.
Rush University Medical Center
Departments of Biochemistry, and Anatomy and Cell Biology
600 S. Paulina St. ACFAC 507
Chicago, IL. 60612
T: 312-932-6780
F: 312-942-3053
carol_muehleman@rush.edu

PARTNERS' NAMES AND AFFILIATIONS:
Klaus E. Kuettner (Rush University Medical Center), Thomas Schmid ((Rush), Jun Li (Rush), Ada Cole (Rush), Zhong Zhong (NLSL, Brookhaven National Laboratory), Miles Wernick (Illinois Institute of Technology) Yongyi Yang (Illinois Institute of Technology), Jovan Brankov (Illinois Institute of Technology), Thomas Irving (Illinois Institute of Technology), L. Dean Chapman (University of Saskatchewan, Canada), Allen Gradzinsky (Massachusetts Institute of Technology), Juergen Mollenhauer (University of Jena, Germany)

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

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

ABSTRACT
The non-invasive detection of early or mid-stage pathological cartilage changes, prior to any bone changes, in degenerative joint disease is of importance so that behavior modification, disease modifying agents, and other treatment regimes may be undertaken in a timely manner. The current gold standard of diagnosis of degenerative joint disease is conventional radiography, a method that addresses only joint space narrowing as a result of cartilage loss and bone changes such as sclerosis and osteophytosis. By this stage, the joint is most likely committed to a pathological progression. Furthermore, at least one study suggests that conventional radiographs are unreliable for evaluating cartilage loss in patients with early OA since, in most cases, joint space narrowing is secondary to meniscal extrusion rather than thinning of cartilage. Diffraction Enhanced Imaging (DEI) is a novel radiographic method, still in experimental stages, that introduces selectivity for the angular deviation of x-rays traversing the subject. It uses a collimated x-ray beam produced by a perfect crystal monochromator. When this beam passes through the subject, a matching analyzer placed between the subject and the detector converts the angular changes in the beam into intensity changes, giving rise to enhanced contrast. Our experiments are carried out at the National Synchrotron Source at Brookhaven National Laboratory, but the DEI technology is not intrinsically tied to a synchrotron and efforts are underway to translate the technique to a compact source of X-rays. We have found that cartilage lesions display as contrast heterogeneities on DEI images. Because the refraction (half points on the rocking curve) images highlight edges, it was here that we are best able to identify lesion outlines and, therefore, determine the severity of a lesion, and whether or not it involves just the articular surface or involves deeper layers as well. Since DEI is a transmission radiographic technique it depicts actual morphology, i.e. the shapes observed on the images are representative of those of the specimen. For instance, surface fibrillation is seen as a roughening of the specimen surface on a DEI image. If the fibrillation is deeper into the tissue, it appears as very small contrast heterogeneities (darker regions as compared to surrounding lighter area) within the depth of the cartilage. A fissure is seen as a contrast heterogeneity in its shape. If a lesion only interrupts a portion of the thickness of the cartilage specimen and does not compromise the full width running parallel to the X-ray beam, the cartilage appears intact in its height but a contrast heterogeneity will be present in the shape as the lesion itself. All lesions can be followed in their entirety through the depth of the cartilage. Using human observer data, we found high correlation between two DEI image readers and the actual grossly observable grade of cartilage degeneration on human tali (on the order of .8) and with a high inter-observer reliability. Several lesions proved to be a challenge in identification if studied only in the anterior-posterior view. For instance, it was occasionally difficult to decipher a Grade 3 erosion from a Grade 4 erosion if much of the

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

STATUS OF RESEARCH AND PARTNERSHIP:

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

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

ISSUES

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

PI: Hunter P. Peckham, Ph.D.
Department of Biomedical Engineering
2500 MetroHealth Drive – Hamann 601
Case Western Reserve University
216-778-3480
pxp2@cwru.edu
http://feswww.fes.cwru.edu/

PROJECT TITLE: Development of Networked Implantable Neuroprostheses.

PARTNERS' NAMES AND AFFILIATIONS:
microHelix, Inc. (Portland, OR), MicroStrain, Inc. (Burlington, VT), CWRU Yeager Center for Electrochemical Science (Cleveland, OH), Synapse Biomedical, LLC. (Oberlin, OH), NDI Medical, LLC. (Cleveland, OH)

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

ABSTRACT
Neuroprosthetic devices that electrically stimulate paralyzed muscles provide functional enhancements for individuals with spinal cord injury and stroke such as standing and stepping, reaching and grasping, and bladder and bowel function. Implanted neuroprostheses have been demonstrated to provide a significant improvement over all existing alternatives for treatment, and some of these systems have now become broadly available. There is a great potential for neuroprostheses to provide even more function, and to impact a much broader range of disabilities. However, one major impediment to further progress is the inflexible architecture of existing implantable neuroprostheses. It is necessary to make a step-change in the design of these systems and progress to an architecture that allows flexibility and scalability so that it can be generalized to multiple applications. In addition, it is important that new neuroprosthetic systems eliminate the need for externally-worn components, while at the same time minimizing the surgical installation and servicing effort. To satisfy these requirements, our Biomedical Research Partnership (BRP) project is developing a networked neuroprosthetic system (NNPS).
The NNPS concept is based on a network of small implanted modules, distributed throughout the body, and linked to a replaceable centralized power source. A variety of modules will be developed, each with a specific function including: muscle-based stimulation, nerve cuff stimulation, biopotential (electromyogram, electro-oculogram, electro-encephalogram, electroneurogram) signal recording, body segment orientation measurement and acceleration measurement. Other potential modules that could be incorporated into this system include mechanical actuators, joint angle transduction, and strain gage based sensors.

STATUS OF RESEARCH AND PARTNERSHIP
The design of our proposed neuroprosthetic system is driven by the functional needs of individuals with central nervous system diseases and trauma. It is designed to provide chronic function for individuals at home and in the community, and is based on the principles of neuroprosthetic use that have evolved over the past 30 years through our clinical delivery of motor neuroprostheses to individuals with spinal cord injury and stroke. We developed functional and technical specifications for the proposed neuroprosthetic system based on our analysis of over 50 different anticipated clinical applications. The functional specifications include: no external components required during functional use, complete flexibility in the configuration of stimulus (output) and sensor (input) channels, ability to expand the system and incorporate new output and input concepts, ability to upgrade the system after initial implantation without component removal, scalable to efficiently meet the needs from simple to advanced system requirements, ease of replacement of failed components, surgical installation with limited incisions, open architecture,

battery lifetime of at least 5 years with a single simple replacement procedure, small size for minimizing exposed surface area, and biocompatibility enabling operation in the body for 50 years. The technical specifications include: 1) from 1 to 68 stimulus channels in any combination of muscle-based and nerve-based electrodes with various stimulation waveforms; 2) from 0 to 25 sensor inputs in any combination of the following modalities: myoelectric, electro-oculogram and electro-neurogram signals; orientation (i.e. position in space) and acceleration; joint angle and surface contact; physical quantities of applied pressure, strain and temperature; 3) multiple wireless inputs from external sensors, including switches, voice and external sensor inputs; 4) data transmission rates between implanted modules of 100 Kb/s; and 5) at least 8 hours of operation per battery charge. The NNPS developed in this project is expected to meet or exceed all of these specifications.

To date we have focused our efforts on those critical aspects of the NNPS design concept that required new knowledge and/or techniques. We have evaluated and modeled several network topologies based upon safety, reliability, performance, ease of implementation, and the ability to upgrade. We have selected a network topology and identified a network communication protocol. We have simulated a physical layer implementation for the internal network that will simplify the segment bus interconnect mechanism. We have selected technologies that are cutting edge, yet are attainable within the scope of the project timeframe. We have assembled the necessary industrial, academic and clinical partnerships in order to complete the design and fabrication of the NNPS.

The specific technical design accomplishments during the past year include the fabrication of a network bench top test system for NNPS analysis. The test system has multiple module and segment capacity, including a graphical user interface for system performance analysis. Feasibility testing of the external network prototype hardware for both the transcutaneous wireless link and the external component wireless network has begun. We have performed mechanical modeling of system components using design automation software. Methods for optimizing the electrical and mechanical implementations of the internal network physical layer infrastructure have been developed. A proprietary networked data-modulation scheme was developed that reduces module power-consumption demands but allows high-speed network data communication. A communication protocol based on the Controller Area Network (CAN) has been developed which includes messages for error handling, network maintenance functions, real time control, data collection, scheduler setup, and scheduler control. We have completed the design of the muscle actuator and myoelectric sensor module circuit and we are preparing for the fabrication of prototypes of these modules for testing.

This project will result in the development of a very powerful, highly flexible and easily scalable neuroprosthetic system. With the basic configuration established, we are now ready to proceed to component development, fabrication and testing, leading to human implantation of the first NNPS during the proposed project time period. The open architecture of the NNPS allows the straightforward incorporation of ideas generated from our research and from research advances made worldwide. We believe that the proposed approach is absolutely necessary to allow both clinical and research advances to continue to take place in this field.

ISSUES

The project is now in a competing continuation.

Our primary issue remains managerial rather than technical. We feel that we have made substantial progress in the research. However, we have encountered delays and problems when attempting to exercise flexibility in changing existing and establishing new contractual relationships with outside industry. An improved ability to quickly and freely team with industrial partners and form more dynamic working partnerships would greatly enable us to evaluate existing technologies and investigate new ones. Clearly communicating the intended flexibility of the NIH-BRP program to University administrative officials – when dealing with subcontractors – would streamline our ability to make changes in consortiums and their related budgets, and thus improve our efficiency and speed progress.

PI: Eli Peli, MSEE, OD
Schepens Eye Research Institute; Harvard Medical School
Schepens Eye Research Institute
20 Staniford Street
Boston MA 02114-2508
T: 617 912 2597
eli@vision.eri.harvard.edu
http://www.eri.harvard.edu/faculty/peli/index.html

PROJECT TITLE: Engineering Approaches to Low Vision Rehabilitation
PARTNERS' NAMES AND AFFILIATIONS: Massachusetts Eye & Ear Infirmary, Boston, MA
Dept. Psychology, Boston College, Chestnut Hill, MA
DigiVision Inc. San Diego, CA
The Lighthouse Inc., New York, NY
Chadwick Optical, White River Junction,VT
University of Cambridge, England
MicroOptical Corp., Westwood, MA
Innovative Visual Rehabilitation, VA Med. Ctr, Boston, MA
Dept. Psychology, Univ. of Groningen, Holland
Belgian Road Safety Institute (BIVV/IBSR), Belgium
Ghent University Hospital, Belgium
Dept. Ophthalmology, Univ. Alabama at Birmingham, AL

GRANTING NIH INSTITUTE/CENTER: National Eye Institute

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, will be 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. Also, we will show that various combinations of these approaches are possible and likely to be beneficial. In our assessment and testing we emphasize two approaches: a virtual environment for controlled and quantitative testing in the laboratory; and on-the-street evaluation for real-life determination 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 well. A paper describing preliminary evaluation of an electronic device - augmented-vision HMD for monocular restricted peripheral visual field - have been published (Proc. SID) and a paper on the evaluation of first prototype HMD for people with night blindness was accepted for publication. The third generation of the HMD was delivered and is undergoing testing. Low weight and low power portable edge detection (mono-polar) devices have been incorporated with the display system. A bi-polar version of the device needed for the dynamic control of display was delivered and has been used in one project. Another novel optical device implementing multiplexing was invented and a patent application submitted. Studies of eye movements while watching TV needed for the dynamic control of displays, have been completed, analysis is underway. The virtual mall is in place and working. Two papers were presented at conferences, a third study is on the way, and a paper on the calibration system

was accepted for publication. Real walking studies with two devices are underway and have completed testing 19 out of 24 subjects. We expect completing these studies this summer. On-road driving studies were completed in Holland and AL and one will start in Belgium soon. The first driving-simulator study will start this summer at the Boston VA. Two papers on the image enhancement projects were published. In the last year we have published 10 journal papers, 3 conference proceedings, and have been awarded a patent. We have presented numerous conference papers including invited and award presentations. An active web site serves both for internal project communications and dissemination of information.

ISSUES

To solve the problem we had with the National Advanced Driving Simulator (NADS) in Iowa, we formed a new partnership with the Center for Innovative Visual Rehabilitation at the VA Med. Ctr. in Boston. For the project we purchased a FAAC driving simulator and our first study is being developed on that simulator. It has proven very difficult to develop useful scenarios for visual performance with devices on the simulator, which was not designed for research. However, we feel that we are achieving our goal of creating a real evaluation tool and not just a computer game.

The MA Registry of Motor Vehicles did not approve the on-road driving studies. So, we formed new partnerships in Europe and AL. The group from Groningen, Holland, performed a hemianopia driving study with our prism treatment that is completed. A second study is planned in Belgium, that awaits resolution of administrative issues. Another partnership forged with the Dept of Ophthalmology at the Univ. of Alabama at Birmingham has resulted in an on-road driving with restricted peripheral field study that has been completed.

Budget reduction for years 4 and 5 imposed by NEI at time of award are limiting our ability to complete our project as necessary and may cause a significant reduction in scope of the project.

PI: Arkady Pertsov, PH.D.
Upstate Medical University
State University of New York (SUNY) Department of Pharmacology,
750 East Adams Street,
Syracuse, NY 13210
T: (315) 464-7986
F: (315) 464-8000
pertsova@upstate.edu
http://web.upstate.edu/perzova/

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

PARTNERS' NAMES AND AFFILIATIONS:
David Weitz, Department of Physics, Harvard University (Boston, Massachusetts);
David Boas, The Massachusetts General Hospital NMR Center (Boston, Massachusetts);
Leslie Loew, UCONN Health Center; Center for Biomedical Imaging Technology (Farmington, Connecticut)

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 imaging using voltage-sensitive dyes was limited primarily to the epicardial surface. The goal of our study is to develop a technology that would enable optical imaging of electrical excitation throughout the myocardial wall. Specifically, this technology should image the filaments, or organizing centers of vortex-like electrical activity. These are widely believed to be responsible for the initiation and maintenance of ventricular fibrillation, and the filaments are a key to their behavior. To address the technical challenges of this new technology we coordinated effort of the research groups of Dr. A. Pertsov, who pioneered the three-dimensional 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
During the first year of support our effort was focused primarily on specific Aims 1 and 3. In particular, we achieved a significant progress in solving the forward problem proposed under Specific Aim 1.

Specific Aim 1. In collaboration with Drs Kerbage, Weitz, and Popp at Harvard University, we completed measurements of light absorption and scattering in myocardial tissues stained with voltage-sensitive dye di-4- ANEPPS. Based on these measurements we developed a two-stage model in which the output of a 3-D ionic model of electrical excitation serves as the input to an optical model of light scattering and absorption inside heart tissue. The model permits unique optical signatures to be obtained

from given 3-D patterns of electrical activity for direct comparison with experimental data, thus yielding information about such activity.

To illustrate applications of the model, we simulated surface fluorescence signals produced by 3-D electrical activity during epicardial and endocardial pacing. We discovered that the morphology of the optical action potential was highly sensitive to the transmural component of wave front velocity and could be used to predict wave front orientation with respect to the surface. The model's predictions were validated in optical mapping experiments conducted at SUNY Upstate medical University. These findings should lead to improved accuracy of the measurements of conduction velocity in ventricular myocardium, which is an important indicator of its physiological condition and of its susceptibility to cardiac arrhythmias and can be used for assessment of pharmacological agents.

Specific Aim 2. Dr. Wellner and Dr. Khait at SUNY Upstate started working on deconvolution approaches for imaging of intramural sources of excitation including scroll wave filaments - the organizing centers of 3D reentrant activity in myocardial tissue, responsible for the most dangerous cardiac arrhythmias. It is expected that by the end of the year they will develop an algorithm for depth detection of filament-like one-dimensional sources.

Specific Aim 3. In collaboration with Dr. Loew Laboratory at the Center for Biomedical Imaging Technology, University of Connecticut Health Center, we have been working on design, synthesis and testing in myocardial tissues a family of near-infrared voltage-sensitive dyes optimized for 3D imaging of electrical activation in the heart. The main focus was on the preparation of new cyclodextrin-encapsulated hydrophobic styryl dyes. The rationale for these dyes is that the cyclodextrin renders them sufficiently soluble that they can be delivered deeply into the cardiac tissue during perfusion. Once they are bound, they will adhere strongly to the outer surface of the myocyte membranes because of their strong hydrophobicity and amphipathic structures. Dye-cyclodextrin complexes for new dyes were prepared. The dyes are now being tested at Dr. Pertsov's laboratory on coronary perfused tissue preparations and on monolayers of cardiac myocytes.

Specific Aim 4. During the an effort has been made to improve the speed of optical recording using second harmonic generation. Dr. Loew and his collaborators have improved their microscope to allow for well registered fast line scanning and synchronization of the line scans with electrical signal. This will permit them to move forward in the coming months with demonstrations of optical cardiac action potentials captured by second harmonic imaging of dye-stained cardiomyocytes. If successful these experiments will significantly improve the accuracy of the measurements of spatial and temporal distribution of the transmembrane potential at a subcellular resolution in cardiac myocytes and other types of excitable cells.

ISSUES: none

PI: Richard D. Rabbitt, Ph.D.
Department of Bioengineering
20 South 2030 East, Room 506 BPRB
Salt Lake City, UT 84112
T: 801 581-6968
r.rabbit@utah.edu
http://www.bioen.utah.edu/faculty/RDR

PROJECT TITLE: Micro-electric impedance spectroscopy (microEIS) of hair cells

PARTNERS' NAMES AND AFFILIATIONS: Baylor College of Medicine, W.E. Brownell (Houston, TX); NASA, R. Boyle (Ames, CA), University of Utah, R.D. Rabbitt, D. Christensen and T. Ayliffe (Salt Lake City, UT); Washington University, S.M. Highstein (St. Louis, MO)

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

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 (e.g. acetylcholine efferent neurotransmitter). Results are contributing to our fundamental understanding of the spatial distribution and temporal response of electrical properties in these important sensory neurons. Perhaps more importantly, microEIT devices developed as part of the research, are providing an entirely new window through which to view the living machinery of a wide variety of normal and pathological cells. The project integrates bioelectricity, imaging, bioinstrumentation, micro/nano-bioesensors, physiological modeling/computation, biomechanics and microfluidics. Devices involve on-chip transport of solutions/pharmaceutics and living cells.

STATUS OF RESEARCH AND PARTNERSHIP
The project is currently in the third year of funding (R01 DC04928, start date: August 2001). All subcontracts were established within the first month of the grant. The scientific and engineering aims of the project are proceeding as outlined in the proposal. We have fabricated 9 unique wafer designs, each including approximately 40 useful microdevices of various sizes and layouts. Due to the small scale of the devices and high interrogation frequencies employed, considerable attention has been devoted to the development of reliable, user friendly, microfluid and electrical interconnects. We have developed a quick-connect fluid-mechanical interface that greatly simplifies practical use of the micro-EI chips. The interface includes on-board RF computer-controlled head-stage FET amplifiers and reference impedances. The 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 at unprecedented temporal speed and spatial resolution. Results demonstrate, for the first time, high-frequency electrical resonances in outer hair cells (OHCs) isolated from the mammalian cochlea. 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 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 licensed for use in automated hematology analysis. In summary, we have succeeded in developing, applying, and translating microEI technology. We are currently applying this new technology to address questions of importance to health and the human condition, with specific focus on sensory hair cells of the inner ear.

ISSUES

We have not experienced any serious technical issues. On the administrative side, a breakdown in communication occurred with the execution of one small subcontract. Although this did not adversely effect completion of the aims of the proposal, the PI recommends inclusion of direct support for administrative personnel in BRP budgets. With regard to the future of BRPs, there are concerns regarding: 1) funding levels for BRP competing renewals, 2) relative importance of IP, 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.

PI: Buddy D. Ratner
University of Washington
Box 351720
Seattle, WA 98195-1720
T: 206-616-9718
ratner@uweb.engr.washington.edu

PROJECT TITLE: Engineered Cardiac Morphogenesis: Stem Cells and Scaffolds

PARTNERS' NAMES AND AFFILATIONS:
Prof. Kim Woodhouse, Univ. of Toronto, Toronto Ontario CANADA
Drs. Robert Vernon and Margaret Allen, Hope Heart Institute , Seattle WA, USA

GRANTING NIH INSTITUTE/CENTER: NHLBI

ABSTRACT
The long term aims of this project are to produce tissue engineered ventricular wall patches for myocardial repair, ventricular assist devices, and eventually replacement ventricles. Our team from academia and industry has expertise in biomaterials, bioreactors, tissue biomechanics, embryonic and somatic stem cells, muscle development, vasculogenesis, extracellular matrix, cardiac injury and regeneration, animal and human heart transplantation. This team will collaborate across three research foci: **1) "Instructive" tissue scaffolds**. Advanced biomaterial fabrication will be used to engineer biodegradable matrices and meshes with controlled pore dimensions, modified with receptor specific molecules. Matrices will be optimized to instruct cell attachment, orientation, migration, proliferation, differentiation, and overall tissue organization. **2) Cell and developmental biology**. Primary and stem cell-derived muscle and vascular cells will be studied on modified scaffolds to determine the optimal conditions for producing functional muscle tissue and vascular networks. Engineered tissues will be subjected to mechanical stresses to direct maturation toward *in vivo* phenotypes. Bioreactors will be developed to implement these requirements on a useful scale. **3) Clinical science and animal models**. Contractile ventricular patches will be tested in an injured heart model. Integration with host tissue and restoration of contractile function will be evaluated. A tubular cardiac assist organ comprised of vascularized myocardium and endocardium will also be developed. The "tube hearts" will be conditioned in pulsatile flow circuits, assessed for mechanical performance in vitro, and eventually grafted into aortas of syngeneic rats for in vivo evaluation. Progress toward these goals should establish design principles necessary for constructing more complex ventricular devices

STATUS OF THE RESEARCH AND PARTNERSHIP
This BRP comprises seven major laboratories at the University of Washington, two at the Hope Heart Institute, one at the Univ. of Toronto. The program is currently in its fifth year of funding.

Status of the Partnership: The partnership is active and robust. The UW laboratories are highly interdisciplinary and include units from both the College of Engineering and the School of Medicine. The participation of the Hope Heart Institute and U of Toronto in this program continues to be enthusiastic.

Status of the Research: Cell Source – Cardiomyocytes: The proliferative capacity of even Day 1 fetal cardiomyocytes is extremely low. After trying for some time without success to achieve a line of proliferating cardiomyocytes derived from mouse embryonic stem cells, experiments were tried with cardiomyocyes isolated by a column gradient method from human embryonic cell cells (line H-7). Cells that proliferate for three weeks or more and demonstrate cardiomyocyte markers have been isolated. These may be the first proliferating cardiomyoctyes that have been discovered. We believe we have identified a major autocrine/paracrine stimulation pathway and an intracellular signalling pathway that controls proliferation of these cells. A manuscript describing these very exciting and useful findings is in preparation. We have also recently followed up on highly publicized studies purporting that

hematopoietic stem cells transdifferentiate into cardiomyocytes after direct injection into infracted hearts. Using genetic models that activate reporter genes in the event of transdifferentiation, we showed that cardiac transdifferentiation does not occur when hematopoietic stem cells are transplanted into infarcts. These findings raise a cautionary note for recent clinical trials prompted by the earlier reports. These data were recently published in Nature. In other experiments, we have attempted to stress adult cardiomyocytes to see if they can be shifted into a proliferating mode. One agent explored in these experiments, a phorbol ester, has induced significantly increased DNA synthesis within these adult cells. We are now studying this DNA synthesis to see if it correlates with increased cell proliferation.

Prompted by reports of others that endothelial cells can be observed to convert to cardiomyocytes under co-culture conditions, we have carefully searched for such phenotypic transitions in freshly isolated chick embryo heart cells. Our findings suggest that while markers for both myocytes and endothelial cells are at times mutually seen, conversion is not actually observed, rather cardiomyocytes were activating vWF expression in co-culture. While conversion did not occur, cell fusion did contribute to a small number of the dual labeled cells. Importantly, these studies clarify that endothelial cell to cardiomyoctye conversion is not likely to be a potential cardiomyoctye source or therapeutic strategy. A manuscript describing these studies is about to be submitted.

A novel class of porous synthetic polymers has been developed that are prepared by template-imprinting soluble microspheres. It has long been known that porous structures can induce an angiogenic response. However, the optimal pore size to induce this response is not known since all the materials explored to date have a broad range of pore sizes. Our polymers are essentially monodisperse in pore size. When implanted subcutaneously in mice for one month, materials with 35 micron pore sizes are found to be potently angiogenic compared to other available porous and non-porous materials. This discovery may allow tissue engineered constructs to spontaneously induce needed blood vessels. Further we have developed a novel photo-polymerization scheme that has enabled the fabrication of well-defined three-dimensional macrostructures made from these porous hydrogels using lithography techniques. This process, currently being evaluated for a patent, will facilitate complex scaffolds with improved access for oxygen and nutrient transport. In addition we have continued efforts with PVA-amino-acid foams made using collodial gas aphrons as well as degradable polyetherurethanes, and are exploring new scaffolds including chitosan-alginate foams and modified hyaluronan gels.

We have also focused efforts on other strategies to provide perfusion conduits in tissue engineered constructs. We have made ultrathin membranes from native fibrillar collagen and evaluated the diffusion of species across these membranes. These can be fabricated into micron scale conduits for perfusion as well as to provide cell alignment. We have also constructed banks of engineered microvessels made from aortic smooth muscle cells. These structures have been used to provide nutrient medium throughout a thick volume of ECM-gel seeded with primary cardiomyocytes and cultured for five days. Unfortunately these very narrow diameter microvessels did not remain patent in these first hybrid organ experiments, with over-proliferation of the SMCs at fault. Two publications have resulted from these studies, however.

We continue work on a cryogenic process to produce drug loaded microspheres as a strategy to deliver growth factors that promote angiogenesis in engineered constructs. We developed our own systems for the production of recombinant VEGF in large quantities for these studies. We now have successfully produced rVEGF loaded PLGA microspheres that deliver bioactive VEGF. This delivery system is now being testing in vivo in an infarcted rat heart model. As an additional possible strategy to deliver GFs or otherwise improve the implant, peri-infarct tissue, we continue to develop an AAV6 vector to encode VEGF165 (angiogenesis), hemeoxygenase-1 (a heat shock protein, and potential cell survival factor) or decorin (to modify extracellular matrix to reduce fibrosis). AAV delivery methods and doses have been optimized and AAV6-decorin experiments are in animals now.

ISSUES

We look to refining and strengthening the team members, projects and specific aims in preparation for a renewal submission.

PI: Perry F. Renshaw
McLean Brain Imaging Center
115 Mill Street
Belmont, MA 02478
T: (617) 855-3750
perry@mclean.harvard.edu

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

PARTNERS:
1. Brain Imaging Center, Behavioral Psychopharmacology Research Laboratory, Developmental Biopsychiatry Research Program, Center for Animal Magnetic Resonance Studies (name not officially approved), McLean Hospital, Belmont, MA;
2. Bioengineering Center, Department of Electrical Engineering and Computer Science, Tufts University, Medford, MA
3. Department of Psychiatry, Boston University School of Medicine, Boston, MA
4. Department of Psychiatry, University of New Mexico, Albuquerque, NM

GRANTING INSTITUTE: National Institute on Drug Abuse (RO1 DA 14178)

ABSTRACT
Magnetic resonance spectroscopy (MRS) and functional magnetic resonance imaging (fMRI) are extraordinarily promising new imaging modalities that are increasing our understanding of the nature of drug abuse and addiction. In March, 1999, the Office of National Drug Control Policy (ONDCP) and McLean Hospital agreed to jointly fund a Varian NMR Systems 4.0 T MR scanner which will be dedicated to substance abuse research at the McLean Brain Imaging Center.
The present BRP application describes a series of ten engineering projects which will enhance the capabilities of this unique magnetic resonance research center to conduct studies of individuals with substance abuse disorders. This research program will involve bioengineering and clinical investigators at McLean Hospital, the Beth Israel Hospital, Tufts University, Boston University, the University of Washington, the University of Oxford, the University of California, San Francisco, and Wayne State University.

Specific projects are summarized below:

1. Objective motion detection and correction in time series fMRI experiments.
2. Optimized phased array coil design.
3. FMRI image registration and signal dropout reduction in brain regions with high susceptibility effects.
4. Functional T2 relaxometry of brainstem and midbrain monoaminergic nuclei.
5. Estimation of cerebral blood flow and volume using dynamic susceptibility contrast MRI.
6. Proton echo-planar spectroscopic imaging at 4 T.
7. Two-dimensional, proton magnetic resonance spectroscopy of amino acid neurotransmitters.
8. Statistical methods for assessing drug effects and confounds in MRS and fMRI studies.
9. Concurrent, high resolution optical imaging and fMRI.
10. Concurrent EEG and fMRI assessment of drug-induced alpha wave activity.

All of the projects listed above have been have been designed to address technical limitations encountered in the course of conducting NIDA-funded clinical imaging studies at 1.5 T field strength. Importantly, funds requested for this BRP will be used exclusively to support the engineering aspects of the research projects.

STATUS OF RESEARCH AND PARTNERSHIP:

1. Our BRP grant was funded by NIDA, with a substantial budget cut, effective 1 September 2001. Of the ten projects listed above, one (#8) was eliminated and two (#2 and #3) were combined. The remaining 8 projects all had significant budget cuts, based primarily on the review of the grant proposal.
2. The Varian Unity/Inova 4 T Scanner was installed at McLean Hospital in May, 2001. We continue to work with Varian to improve the performance of the scanner for human clinical studies and for non-human primate studies. Many unresolved issues reported in 2002 (scanner quenching, patient table, sound dampening and filters for decoupling) have been resolved. Remaining issues include: software for decoupling, EPI stability and ghosting, and user interface.
3. In Year 1, additional funding to support hardware purchases was obtained from the Counterdrug Technology Assessment Center (CTAC) of the Office of National Drug Control Policy (ONDCP) to expand the scope of the work that we could do within the BRP. Equipment has been ordered and some has been delivered. Work on projects #9 and #10 was delayed due to long lead times for equipment delivery.
4. We have performed several upgrades to the 4 T scanner, including installing a higher power RF amplifier to improve spectroscopy performance, obtaining a new shim amplifier to enable dynamic shimming, and developing improved reconstruction software in-house.
5. A research agreement was established with Qualisys Medical to supply two near infrared, high resolution cameras for project (#1), improving resolution tenfold over our previous cameras.
6. Methods for 2D MRS on the 4 T scanner continue to be used (project 7).
7. Last year, project 6 moved from Wayne State University to the University of New Mexico.
8. Four new areas of research evolved in Year 2: carbon-13 MRS studies of cerebral metabolism , biological effects of magnetic stimulation, visual psychophysics studies using fMRI, and sodium-23 MRS studies of cerebral membrane properties. In Year 3, project 10 was discontinued due to the inability to successfully recruit a replacement for the PI, and four additional research projects were identified: fMRI studies in neuropathic pain, PET imaging of brain monoaminergic systems, and non-human primate studies in awake-restrained squirrel monkeys (stimulant abuse and treatment studies) and cynomolgous monkeys (opiate analgesia/addiction studies).
9. Over the past year, we have been able to obtain additional funding for this research program from GlaxoSmithKline (to support the 2D MRS and the ^{13}C MRS projects), the Counterdrug Technology Assessment Center/Office of National Drug Control Policy and the National Center for Research Resources (to support the purchase of a dedicated, 9.4 T animal scanner), and Descartes Pharmaceuticals (to support the lease of an fMRI dedicated 3 T Siemens Trio scanner). As our initial grant proposal was only funded at 50% of the original request, we have actively sought supplemental funding to increase the scope of this research.

ISSUES:

No input.

PI: H. Grady Rylander
The University of Texas at Austin
Eng Sci Bldg R617C
Austin, Texas, 78712
T: (512) 471-1995
F: (512) 471-0616
rylander@mail.utexas.edu

CO-PI: Thomas Milner
The University of Texas at Austin
Eng Sci Bldg R 20A
Austin, Texas, 78712
T: (512) 471-1332
F: (512) 471-0616
milner@mail.utexas.edu

PROJECT TITLE: Polarization Sensitive Optical Coherence Tomography (PSOCT) for Glaucoma Diagnosis

PARTNERS' NAMES AND AFFILIATIONS: Johannes de Boer, Barry Cense, Teresa Chen, Hyle Park (Harvard Medical School and Wellman Laboratories of Photomedicine, Massachusetts General Hospital). deboer@helix.mgh.harvard.edu

GRANTING NIH INSTITUTE/CENTER: NIBIB

ABSTRACT:
The goal of our project is to measure depth resolved birefringence of the retinal nerve fiber layer (RNFL) and study how the birefringence changes with glaucoma. We have constructed two PSOCT systems: open-air and fiber-based systems at UT Austin and Massachusetts General Hospital, respectively. The fiber-based PSOCT system is coupled to a slit lamp for clinical measurements. The open-air PSOCT system is used to study glaucoma in a primate model.

STATUS OF RESEARCH AND PARTNERSHIP
Accomplishments this year include improving the acquisition speed of the clinical instrument by switching to a spectral domain OCT system and implementing a multiple incident state non-linear algorithm for estimating phase retardation (PR). RNFL birefringence measurements have been recorded from primate subjects at UT Austin and glaucoma patients and normal individuals at MGH.

Multiple maps have been measured for normal primate peripapillary RNFL thickness (RNFLT), PR, and PR/UD (birefringence). RNFLT varies from 20μ to 200μ and exhibits a strong radial gradient. RNFLT is highest in the superior and inferior bundles near the optic nervehead. Absolute values of RNFLT are very sensitive to optic nervehead registration. Single-pass phase retardation varies from 2° to 35° and is highly correlated with RNFLT. Phase retardation (PR) is highest in the superior and inferior bundles and lowest in the nasal and temporal bundles. Radial gradient in PR is less than RNFLT. Birefringence (proportional to PR/UD) varies from 0.02 °/μm to 0.3 °/μm and is highest in the superior and inferior bundles. Radial gradient is less for PR/UD than RNFLT. Birefringence is statistically higher in the superior and inferior quadrants compared with the nasal and temporal quadrants. The average birefringence (at 820 nm) in the superior and inferior quadrants was 17.38 °/100μm or $\Delta n = 4$ (10^{-4}) and average birefringence in the nasal and temporal quadrants was 6.09 °/100μm or $\Delta n = 1.4$ (10^{-4}).

We measured the birefringence of the normal human retinal nerve fiber layer *in vivo*. Birefringence of healthy RNFL was found to be constant as a function of scan radius but varies as a function of position around the ONH, with higher values occurring superior and inferior to the ONH. Measured double pass phase retardation per unit depth values around the ONH range between 0.10 and 0.35 °/μm, equivalent to birefringence values of $1.2 \cdot 10^{-4}$ and $4.1 \cdot 10^{-4}$ respectively, measured at a wavelength of 840 nm.

RNFLT and PR are measured independently although they are highly correlated. The principal investigator and colleagues hypothesize that a discriminant function employing both measures may be superior to either alone in differentiating glaucoma progression.

Although existing diagnostic technologies like scanning laser polarimetery (GDx) measure PR and give useful information about the RNFL, the results should not be interpreted as RNFLT. To determine RNFLT from measured RNFL phase retardation, local birefringence must be known. We have shown that birefringence is not constant across the RNFL. Measurements with the GDx should be combined with other objective measures to discriminate glaucoma progression.

In primates we have observed that both PR and birefringence have a smaller radial gradient than RNFLT. This property may help in longitudinal studies where sequential registration is important in following glaucoma progression. It is unknown at this time, but important to study, how the PR and PR/UD maps change with glaucoma progression.

In the past year we developed a new approach for Optical Coherence Tomography that is inherently more sensitive than previous approaches. We realized a 150 fold improvement in sensitivity over current state of the art OCT technology allowing video rate *in vivo* retinal imaging without compromising image quality. Our video rate (29 frames/sec) spectral domain OCT (SD-OCT) system can continuously obtain *in vivo* images of the human retina at 29,300 A-lines/s. A 3-D volume of the retina was constructed from the data acquired. Sensitivity was 98.4 dB and the axial resolution was 6 μm. Using Doppler signal processing, bi-directional flow was measured in retinal artery-vein pairs. The ultra-high-speed SD-ODT system allows visualization of the pulsatile nature of retinal blood flow, detects blood flow within the choroid and retinal capillaries, and provides information on the cardiac cycle. Using a new broadband superluminescent source, we presented the first experimental verification of *in vivo* axial resolution of 3.5 μm by analyzing the specular reflection form the foveal umbo.

A fiber-based differential phase optical low coherence reflectometer has been built that can resolve one milliradian or an optical pathlength difference of one angstrom. Optical measurement of neuronal swelling that accompanies the action potential has been made non-invasively *in situ*. Transient neural surface displacement in the walking nerve of the crayfish is about 1 nm in amplitude and coincident with action potential arrival at the optical measurement site. This technique could be applied in the eye to measure the swelling in the RNFL that accompanies action potential propagation.

Our partnership conducts monthly videoconferences to share information and research directions. Both groups share PhD students to facilitate communication. At both institutions, weekly group meetings are held with a formal presentation followed by questions.

ISSUES

1. Change in research focus as the project evolves
2. Delays caused by technical problems
3. Guidance with clinical trials

PI: Chris J. Sackellares, M.D.
University of Florida
PO Box 100244
Gainesville, FL 32610-0244
sackellares@mbi.ufl.edu
http://brp.mbi.ufl.edu

PROJECT TITLE: Bioengineering Research Partnership for Brain Dynamics

PARTNERS' NAME AND AFFILIATIONS:

University of Florida: Paul R. Carney, M.D.; Jose C. Principe, Ph.D.; Pardalos M. Pardalos, Ph.D.; Mark C.K. Yang, Ph.D.; Deng-Shan Shiau, Ph.D.
Arizona State University: Leon D. Iasemidis, Ph.D.

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

ABSTRACT
Epilepsy is a common neurological disorder that causes spontaneous recurrent seizures. In spite of major advances in pharmacology, neuroimaging, clinical neurophysiology, and neurosurgery, many patients remain disabled due to uncontrolled seizures. We propose to develop novel diagnostic and therapeutic tools, based on recent discoveries regarding dynamical mechanisms initiating epileptic seizures. We have found characteristic preictal dynamical changes, detectable in the electroencephalogram (EEG), preceding seizures by over 30 minutes (preictal transition, PT). More recently, other investigators have confirmed the presence of PT. Our research indicates that the PT is demonstrable in the EEG in approximately 90% of seizures and that automated paradigms can be used to predict seizures. The potential to predict seizures in advance provides an opportunity to develop innovative diagnostic and therapeutic approaches. Our specific aims are: **(1)** To continue the development of dynamic measures for the quantification of the spatiotemporal properties of the epileptic transition; **(2)** To develop specific pattern recognition algorithms for a seizure warning system (SWS) based upon the on-line features of the dynamical properties of brain electrical activity; **(3)** To implement the dynamic features and pattern recognition algorithms in a SWS for on-line, real-time detection of the preictal dynamical transition; and **(4)** To evaluate the effects of therapeutic interventions during the preictal transition.
We plan to continue pursuing the goals of Specific Aims 1-4 during the upcoming year. We will focus on the improvement of pattern recognition algorithms SWS, pilot study of implementing SWS for on-line real-time monitoring (Amphiarus 1), and therapeutic interventions in rodent model. Amphiarus 1 uses a program structure such that any new measure can be incorporated as a subroutine. Alternatively, multiple dynamical measures can be used to define the dyanamical state, using appropriate statistical comparisons to detect preictal convergence among electrode sites. We will further explore the parameter space of Amphiarus 1. In addition, we plan to test the algorithms on larger datasets. These datasets will include scalp and intracranial recordings in patients with focal onset epilepsy. In addition, we will test and improve the algorithms in the rodent models. We anticipate evaluating intervention protocols in the animal models toward the end of the upcoming 4th budget year.

STATUS OF RESEARCH AND PARTNERSHIP
Specific Aim 1: Development of new measures for quantification of the directional information flow in the brain continues in the Arizona State University Brain Dynamics Laboratory (ASBDL). Two measures have been developed: the cross-Lyapunov exponents and the transfer of entropy. Initial results indicate that determination of the directional information flow is more useful than the net flow. ASBDL also continues on the development of mathematical models, based on the theory of coupled chaotic oscillators, for the modeling of the dynamical characteristics of the epileptic transition. A multivariate AR approach in the state space has been developed. This modeling approach continues to provide new insights into the understanding of the route of the epileptic brain to and out from a seizure, as well as into our previous claim of seizures as resetting mechanisms of the brain. The University of Florida Computational Neuro-Engineering Laboratory (UFCNEL) has developed a more efficient algorithm for calculating dynamical measures. The approach reduces the computational complexity drastically by exploiting the accurate quantization properties of the self-organizing map (SOM) in representing the dynamics of the signal in the phase space. This new algorithm will provide an advantage for on-line, real-time applications that are used to detect or predict seizures from EEG signals. It may become particularly important for development of

implantable seizure prediction devices.

Specific Aim 2: The University of Florida Brain Dynamics Laboratory (UFBDL) and ASBDL continue to refine and test seizure prediction algorithms to find optimum methods for detecting the spatiotemporal patterns of the preictal transition. A major advance was improving the performance of seizure prediction algorithm by utilizing adaptive prediction thresholds based upon the dynamical states. The algorithm has been tested and statistically evaluated in a previously recorded library of continuous long-term intracranial and scalp EEG recordings in 17 patients with temporal lobe epilepsy (a total of 239 seizures over 132.38 days). The evaluation method compared the areas above ROC curves from the AWA with the ones from naïve statistical-based prediction schemes. The result from 17 test patients showed that our prediction method is significantly better ($p < 0.01$) than the other naïve methods. We also tested seizure prediction algorithms employing other dynamical measures developed at ASUBDL and UFBDL. For example, the angular frequency algorithm was first tested in computer-generated data from coupled nonlinear oscillator models. Subsequently, it was tested in EEG datasets at the UFBDL. The algorithm performed well, giving a very good sensitivity to false predictions. Another ongoing project is to develop an automated artifact rejection algorithm. Our experience on eye balling the outputs of our seizure prediction algorithms over long-term monitoring EEG shows that about 10% of the false positives are directly related to noise in the signals. Therefore, artifact rejection is a must for robust prediction and detection of epileptic seizures. UFCNEL has developed a novel artifact rejection algorithm based on the Blind Source Separation and Independent Component analysis. This algorithm has been tested on both our prerecorded scalp and intracranial EEG recordings. We envision that the false predictions will be greatly reduced by incorporating this method into our seizure prediction algorithms.

Specific Aim 3: To accomplish real-time seizure prediction and warning, the analysis must take place on-line with the EEG acquisition recording system which requires the data interface between the recording system and the prediction software application. A computer program, Amphiarus-1, incorporating our seizure prediction algorithm has been written in C++ and was tested in the EEG lab at Gainesville VA Hospital by interfacing with Long-term Monitoring System (Nicolet BMSI™ 6000). The software testing was accomplished without the involvement of a patient by simulating multiple recording sessions over a 3-day period using a sine wave generator as the signal input. More continuous on-line real-time runs at the University of Florida Epilepsy Monitoring Unit will allow us to detect other problems or desired enhancements to incorporate into the prototype. This system pilot test of the hardware configuration and the software integration was successful and demonstrates the realization of on-line real time seizure prediction and warning in the clinical setting is now feasible and we are ready for clinical testing using patients with continuous recording over days to further evaluate and improve on the prediction algorithm and software package.

Specific Aim 4: In preparation for trials to test preictal interventions in rodent models, we are conducting experiments to define the seizure characteristics of the animal models. The Chronic Limbic Epilepsy Model (CLE) has been adopted to create epileptic rodents. This spontaneous seizure model is created by inducing prolonged seizures (status epilepticus). The method used in our animal laboratory is electrical stimulation of the hippocampus. After a period of several weeks to a month of recovery, the animals begin to have spontaneous seizures that last for the rest of their lives. To date, 110 clinical seizures in eight rats have been identified and studied. Continuous long-term (weeks) EEG recordings were saved for the analysis of perfecting seizure warning/prediction algorithms in this model. Evaluations of the seizure warning algorithms in pre-recorded EEG are currently underway. We have begun to set up an Amphiarus warning system in the in-vivo laboratory. This will allow us to test the seizure warning system on-line in real time. In addition, we are beginning to investigate the effects of stimulation on dynamical measures of EEG in this rodent model. This will lead us to investigate the effects of therapeutic interventions during the preictal transition.

ISSUES

Progress in development of the physical system and software required for a practical, clinically relevant implementation of Amphiarus seizure-warning algorithms has been more rapid than expected. In the near term, the performance of the existing computers and EEG/video recording storage system will be a limiting factor. We need to identify funds to update or replace outdated equipment.

PI: David J. Sahn, M.D.
Oregon Health & Science University
Pediatrics (Cardiology)
3181 SW Sam Jackson Park Road, L608
Portland, OR 97239-3098
T: (503) 494-2191
F: (503) 494-2190
sahnd@ohsu.edu

PROJECT TITLE: High Frequency Ultrasound Arrays for Cardiac Imaging 5-R01-HL067647-03

PARTNERS' NAMES AND AFFILIATIONS:

Matthew O'Donnell, University of Michigan, Ann Arbor, MI
Douglas Stephens, University of California, Davis, Davis, CA
Kai Thomenius, GE Corporate R & D, Schenectady, NY
K. Kirk Shung, University of Southern California, Los Angeles, CA
Raymond Chia, Irvine Biomedical, Inc. Irvine, CA
Pierre Khuri-Yakub, Stanford University, Stanford, CA

ABSTRACT
Among the most prolonged and detailed interventional catheterization procedures, electrophysiological (EP) mapping and radiofrequency (RF) ablation for atrial and ventricular arrhythmias has received recent attention because of the now well-recognized need for spatial mapping in addition to fluoroscopic catheter localization. The complexity and numbers of the procedures being undertaken is increasing. We propose to design, develop and test a family of 2D and real-time 3D intracardiac ultrasound imaging devices which, at 9-15MHz operating frequency, will provide spatial localization, and both tissue velocity and strain rate estimates of mechanical activation in atrial and ventricular walls, to guide electrical mapping and ablation. This should greatly shorten imaging time to localize critical areas. Our devices will be integrated with the EP and RF ablation electrode so that they can visualize the lesion, anatomically monitor the ablation procedure and map the distribution of temperature during RF delivery.

STATUS OF RESEARCH AND PARTNERSHIP
Advanced technology for intracardiac ultrasound for guiding anatomical catheter based interventions and especially electrophysiologic mapping and ablation obviates the need for transesophageal echo and yet provides a level of anatomical detail that should assist the performance of these complex catheter-based procedures and shorten fluoroscopy time. As a first device, developed from our BRP as proposed, we have completed design fabrication and initial testing of a side-looking 64-element phased array on an EP enabled catheter which already, with first prototypes, appeared to exceed penetration and resolution of currently available products and yet make the ability to sense and/or perform ablation on the same device. Preliminary animal experiments show that we can provide high detail for anatomic visualization and use tissue Doppler and advanced strain rate methods for determining mechanical contraction and propagation as guides to the location of arrhythmias. This also opens the area of guidance for resynchronization using this intracardiac technology in addition to ablation for arrhythmias. The present side-looking array is undergoing safety and efficacy for determination of an IDE from the FDA to allow human testing at the end of this year. The second device we build will be a forward-looking 9 French catheter either with a 32-element piezo-electric or potentially a CMUT array. A CMUT approach has many advantages as it can be implemented with miniaturized pulsars and/or multiplexing and can be adapted by varying the DC voltage to a variety of frequencies and/or power output. Theoretically, this second device should be able to generate high intensity ultrasound, or HIFU, so that not only electrical RF ablation but also high frequency ultrasound ablation (which is more appropriate for ventricular arrhythmias) can be performed

under imaging guidance. The third device, a 9 French catheter with a 3D imaging ring, has also been modified so that the available lumen will also admit a 7 French steerable catheter. The 3D imaging, forward-looking ring array, which will be CMUT based, has been moved to the outer tier. The ablation surface is now on the side of the tip of the catheter. The potential implementing both forward-looking micro linear array and the ring array as CMUT technology elevates our goals toward even more advanced technologies.

ISSUES

Fiscal:

The fabrication of the first piezo-electric arrays for the hockey stick and connectors and cabling for those devices have helped us clear some carryover that occurred in the first year when JOMED was withdrawn. Our yearly budget was adjusted to accelerate work on advanced strain rate imaging by Matt O'Donnell and an additional sub-contract to Khuri-Yakub for development of the CMUT version of the forward looking ring array. There is still a significant carryover at the end of the second year, which will be directed towards fabrication of enough hockey stick side-looking arrays.

Science and Organizational:

Our work has been preliminarily presented in abstract form, both at the American Heart Association 2003 and at the American College of Cardiology in 2004 and has drawn significant interest both from the ultrasound and the EP communities. Six abstracts were submitted to the American Heart Association for the 2004 Scientific Sessions next November. Organization of the partnership itself is solid; interaction is enjoyable, collegial and with Doug Stephens as engineering coordinator and using our FTP site we are moving forward efficiently. The partnership meeting along with our advisory board is scheduled for late summer and will occur shortly after the BRP meeting in Washington, D.C.

P.I.: Dr. Guy Salama, Ph.D.
Department of Cell Biology and Physiology
University of Pittsburgh, School of Medicine
Room S 314 Biomedical Science Tower
200 Lothrop Street
Pittsburgh, PA 15261
T: 412-648-9354
F: 412-648-8330
gsalama@pitt.edu

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

PARTNERS' NAMES AND AFFILIATIONS:
Alan Waggoner and Lauren Ernst, Carnegie Mellon University, Pittsburgh, PA
Fred Lanni, Carnegie Mellon University, Pittsburgh, PA

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

ABSTRACT
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 Ca2+ 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 Center for Light Microscope Imaging and Biotechnology (CLMIB)) and Lauren Ernst will develop optical probes (voltage-sensitive and Ca2+ indicator dyes) with long excitation and emission wavelengths to improve tissue penetration and reduce light scattering from the myocardium. Dr. Fred Lanni (at CLMIB) 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): Two approaches will be developed and tested to obtain the best possible HSDRI system. (a) A system based on a Ronchi line grating to focus dark and bright bands in a focal plane 2-5 mm deep in ventricular tissue. Fluorescence images from the tissue will be taken (at 3k frames/s) during shifts of bright and dark bands of light excitation by 1/3 period. Images will be processed on-line to eliminate light emanating above and below the plane of focus to obtain depth-resolved images, at 1k frames/s. (b) A standard Nipkow spinning disk confocal imager will be modified for large fields-of-view (3x3 mm2) and high frame rates.
Aim 2: Drs. Waggoner and Ernst will synthesize new longer wavelength fluorescent dyes to monitor action potentials (APs) or cytosolic free Ca2+ (Cai) and Dr. Salama will test, analyze the spectral characteristics and response characteristics of the new probes in heart muscle.
Aim 3 (Salama, Choi and Lanni): Software will be developed to drive the HSDRI, analyze APs and Cai transients 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. APs will be mapped in 3-D to resolve PV delays during antegrade and retrograde conduction, normoxic and ischemic in paced and during 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 Ca2+ 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. We focus here on the heart because therein lies salient problems that are ready to be addressed by this new technology. However, the wide range of possible applications may lead to the commercialization of this new technology.

STATUS OF RESEARCH AND PARTNERSHIP:
In the first year 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 cm2), 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 □s) and spatial (100x100 □m2) resolution, yielding APs with 40/1 S/N ratio. Software has been developed to map activation and repolarization patterns for hearts under sinus rhythm, pacing protocols and during fibrillation. Maximizing the S/N ratio and learning how to trigger image acquisition were important to build the 3-D imager based on the CMOS camera and an oscillating Ronchi grating. With our partner, Dr. F. Lanni, we are building a 3-D imager for large fields of view (2x2 cm2) that can resolve images 3-5 mm deep in the myocardial wall. The high speed CMOS camera will allow us to achieve our goals of depth resolved images at depths (5 mm) and speeds (> 1K frames/s) to map impulse propagation from the conduction system of the heart to the myocardium and from the endocardium to epicardium.
Another critical aspect of 3-D imaging of electrical activity is the design and synthesis of new optical probes of membrane potential that have longer wavelength characteristics in their excitation and emission spectra. The goal is to synthesize voltage-sensitive dyes that have greater sensitivity to changes in membrane potential (higher □F/F ratio per AP) and function at longer wavelengths to improve depth of penetration of light and reduce light scattering by the tissue. Our partners, Drs. A. Waggoner and L. Ernst have made a set of 5 new probes that can be excited at ~ 700 nm and emit at ~ 850 nm. One of these dyes, Pittsburgh 1 (PGH1) has been found to have twice the sensitivity to voltage compared to the best currently available probe (di4-ANEPPS) and can be excited at 690 nm with a peak emission at 850 nm. Optically recorded APs were found to be stable over several hours and to exhibit the shape and time course of APs recorded with intracellular microelectrodes. High speed spectral measurements of the 'Action Spectra" (voltage-dependent spectral changes) were measured with a linear CCD array that records a spectrum in 20 ms was used to optimize the optical components needed to map APs in 3-D. New dyes are currently being tested under different staining conditions by varying the vehicle to maximize the voltage signals that can be obtained at long wavelengths. CMOS images of hearts during fibrillation were of excellent quality allowing us to map the creation and annihilation of reentrant circuits and to map the distribution of sites responsible for the creation of new daughter waves. Rigorous analysis of wavebreak sites indicate that their locations are random and that they do not preferentially occur over large coronary vessels on the heart.

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 would like to discuss is how to provide these new compounds to other investigators and to best serve the scientific community as we develop better dyes and instruments for electrophysiology.

PI: William Shain, PhD
Wadsworth Center
PO Box 509
Albany NY 12201-0509
T: 518-473-3630
shain@wadsworth.org

PROJECT TITLE: Brain Prostheses: Tissue Integration & Compatibility

PARTNERS' NAMES AND AFFILIATIONS:
J.N. Turner, Wadsworth Center (Albany NY), M. Isaacson, Cornell University (Ithaca NY) and University of California, Santa Cruz (Santa Cruz CA), W.M. Saltzman, Yale University (New Haven CT). D.R. Kipke, University of Michigan (Ann Arbor MI), J. Williams, University of Wisconsin (Madison WI), D. Moran, Washington University (St. Louis MO), L. Margolin, FHC (Bowdoinham ME)

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

ABSTRACT
Nanofabricated neural prosthetic devices provide tremendous potential for furthering our understanding of central nervous system (CNS) function and treating CNS disease and injury. Such devices will permit precise localization of targets and control of electrode function. However, the success of these devices is presently limited by reactive biological responses. These experiments are designed to compare and contrast events underlying early and prolonged responses observed following prosthesis insertion in order to develop strategies for successful design and use of neural prosthetics. We will identify the signaling events that produce these responses, identify the source of the signals, and use technology advances in prosthesis insertion, prosthesis design, and pharmacological delivery, to control these events. Reactive responses will be described using our procedures to assess biochemistry and function of individual cells in complex 3-D samples. Confocal microscopy of thick sections (~100 □m) is coupled with immunohistochemical and fluorescence in situ hybridization to permit identification of neurons, astrocytes, and microglia, to measure cytokine production, activation of second messenger signaling pathways, elaboration of extracellular matrix components, and other specific cell products. These techniques permit simultaneous evolution of changes in cell morphology and tissue distribution as well as the relative abundance of messenger RNAs or gene products. We will determine the cells participating in reactive responses, describe cell-cell and cell-prosthesis interactions, and, with our data analysis capabilities, quantify these observations. Cell and organotypic cultures, developmental staging, and mouse genetic models will be used to test experimental hypotheses developed from observations in adult rats. Prostheses will be made using nanofabrication techniques; surfaces will be modified by chemical, biochemical, and physical methods. Pharmacological interventions will be tested by systemic application as well as incorporating microfluidic elements into prosthetic devices. Results from these experiments will provide important new information for the intelligent design of improved biomaterials and micro-devices to control dynamic biological events in the CNS and insure the successful long-term performance of neural prosthetic devices.

STATUS OF RESEARCH AND PARTNERSHIP
Continued analysis of cell participation and signaling around inserted single shank devices has allowed us to hypothesize that activated cells produce signals that diffuse into the tissue forming “zones of influence”. This concept can be used to describe the volume of tissue that must be affected by local drug release for effective drug-delivery. We have developed intervention strategies to control immediate tissue damage due to insertion, early inflammatory responses, and signals maintaining the sustained responses. We studied sustained responses for > than 8 months in rats and >6 months in non-human primates. We

have developed new methods for assessing immediate tissue damage including measurements of cell damage by nuclear staining with cell impermeant dyes, e.g. propidium iodide, vascular casting, and changes in neuron nuclear morphology. These methods have allowed us to assess the degree of damage using different types of devices and different insertion methods. We are now using quantitative assessments of these changes to measure the effectiveness of different drug intervention and insertion strategies. Devices with different numbers and spatial distribution of shanks were used to test the hypothesis that each shank will generate a zone of influence and that, depending on spacing, these will collide producing regions of greater biological response. Spatial-dependent effects were observed. When devices with inter-shank intervals of 250 □m were used, increased reactive responses were observed around the entire device at times corresponding to both early and sustained responses. These results indicate that intervention strategies may be particularly important for controlling cell responses around these devices. We are also taking advantage of the larger volumes of tissue involved in reactive responses to make biochemical analysis feasible. Thus, we are able to describe changes in signals, e.g. cytokines, and activation of signal transduction pathways, e.g. mitogen-activated protein kinases (MAPks). Devices with single long, e.g. 5-mm, shanks were used to determine if different brain regions produced similar reactive responses. These devices were inserted through the cortex and hippocampus and into the thalamus. A similar pattern of reactive responses was observed in all three regions, but region-specific changes in the strength of the responses in astrocytes (glial fibrillary acidic protein (GFAP)-immunolabeling) and microglia (CD11b-immunolabeling) were observed. New fabrication strategies produced improved on-device microfluidic channels. Increased cross-sectional areas were used to improve fluid flow and devices permitting perfusion through the channels have been designed, fabricated, and tested in agar brain phantoms. Microfluidic devices are being used to study immediate cell damage following device insertion as wells as to deliver water-soluble drugs and proteins. We are testing our hypothesis that diffusion, driven by the chemical concentration gradient between the channels and the tissue, is sufficient to deliver drug to the volume of tissue containing the cells releasing signals. Using both small molecules (Hoescht stains, molecular weight=635) and proteins (Texas Red-labeled transferrin, molecular weight=70,000) diffusion-driven release results in movement of molecules as far as 400 □m from insertion sites. This distance is sufficient to provide drugs to the zones of influence described for immediate and sustained reactive responses. While the maximum early sustained responses can extend well beyond this, cells responsible for initiating this response are located well within the drug-delivery zone. Experiments to investigate how changes in device design can effect tissue responses have been initiated. These experiments will correlate electrophysiological measurements with histological results, allowing us to assess ongoing device function, effectiveness of drug delivery, and corresponding cell and tissue responses.

ISSUES

One of our major issues has been the need to extend the range of research activities necessary to maintain our progress in understanding and controlling reactive cell responses. In order to satisfy these needs, we have proposed to increase our partnership and to develop additional collaborations. To this end in our competing renewal, we have increased the numbers of partners and we have also developed collaborative activities with other laboratories including that of A. Schwartz (University of Pittsburgh, Pittsburgh PA), S.J. Kim (Seoul National University, Seoul, South Korea), and the Center for Neural Communication Technologies (CNCT; University of Michigan, Ann Arbor MI). These collaborative activities are a result of the need for inter-disciplinary expertise and facilities to address the complex issues associated with device design and fabrication, and biological assessment of device function, all within the framework of developing devices for routine clinical application. While gathering the scientific staff is relatively easy, orchestrating and administering the necessary communication is a challenge.

PI: Thomas C. Skalak, Ph.D.
University of Virginia
Department of Biomedical Engineering
Box 800759 Health System
Charlottesville, VA 22908
T: 434-924-0270
F: 434-982-3870
tskalak@virginia.edu
www.bme.virginia.edu

PROJECT TITLE: Integrated Control of Vascular Pattern Formation

PARTNERS' NAMES AND AFFILIATIONS:
Gary K. Owens, PhD (Molecular Physiology, University of Virginia)
Richard J. Price, PhD (Biomedical Engineering, University of Virginia)

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

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

STATUS OF RESEARCH AND PARTNERSHIP
To directly assess the role of PDGF □-receptor signaling in arterialization, we are in the process of generating SMC targeted PDGF □-receptor knockout mice. We have obtained heterozygous knockout mice that contain the SM MHC cre transgene, and one floxed PDGF□ receptor allele (which has undergone cre recombination), and these are currently being backcrossed to obtain homozygous SMC targeted knockout of the PDGF□-receptor. A major limitation in the field has been the inability to definitely identify SMC lineages since these cells may show transient loss of typical SMC markers like SM MHC during the remodeling process. To circumvent this limitation, we have completed development of a unique mouse model for studying the origins of SMC that invest newly formed arterioles. In brief, we have crossed our SM MHC cre recombinase transgenic mouse with a floxed ROSA lacZ mouse that shows cre dependent activation of a lacZ indicator gene, thus

providing a model for definitive SMC lineage tracing. We are in the process of using these mice to define the origins of SMC within newly invested arterioles during development (Aim 1) as well as vascular remodeling and patterning in adult animals in response to hypoxia (see Aim 2) and with tumor angiogenesis.

We published new findings (Peirce et al. 2003) on the application of temporal and spatially specific growth factor stimuli, specifically VEGF and Ang-1, to window chambers, with subsequent quantification of the vessel patterning responses. A remarkable result was that VEGF produced increased total length density with an inflated capillary/arteriole ratio while Ang-1 produced increased length density while preserving the normal ratio of capillary size vessels to order 2 vessels (terminal arterioles). This suggests an explanation why VEGF trials in past collateralization studies have not worked optimally, because the VEGF alone produces vessel patterns lacking the proper hierarchical relationship of arterioles to capillaries.

A new direction taken in Aim 1 and Aim 2 overall was to study the lineage of endothelial and smooth muscle cells that participate in angiogenesis and arteriolization following prazosin and hypoxia stimuli. The goal here is to determine whether reports of bone marrow derived stem cell's (BMCs) role in larger artery remodeling are germane to the events occurring in microvascular patterning in the adult. In the models used in this study BMCs have almost no direct role in supplying new endothelial or smooth muscle cells; however, a large number appear to be resident in the tissue, many with pericyte morphology. Another new direction is the discovery (Murfee in review, 2004) of arteriolar and capillary specific pericyte marker, NG-2, a proteoglycan previously known to be expressed in nerves and some vascular cells, but not in a vessel-phenotypic way. As such, NG2 may emerge as a more powerful marker of adult vessel type than the ephrins, previously described by others. The key issue now is whether NG2 is a cause or effect of hemodynamic or other epigenetic factors.

We published the first findings on the new automata simulation of vessel assembly and patterning (Peirce et al, 2004), including the placement of a microbead point source of VEGF for initial capillary development and the later production of PDGF by new vessels with subsequent recruitment of perivascular cells that differentiate into smooth muscle phenotype. Results show good predictive power up to 14 days. This finding is the first demonstration that a cell-based model can independently predict pattern formation in vivo. The paper received the Cover Illustration of FASEB Journal, and was featured in MIT's Technology Review. The method has now also been applied to a number of additional issues in vascular patterning, such as the role of extracellular matrix as a reservoir for growth factors and the role of BMCs in adult remodeling.

ISSUES

Teamwork in managing new discoveries and their follow-up with functional studies continues to be the major issue. Last year, stem cell homing work was initiated, and this year the new discovery of a microvessel arterio-venous polarity marker (NG2) provided a number of new directions requiring careful functional studies and/or chronic cell lineage studies. We manage these with a variety of face to face meetings among PI and co-PIs, post-docs, and graduate students working on the projects. We find that this generates rapid follow-up studies, clear definition of responsible authorship, and often the impetus for independent research careers of the involved post-docs or students who have the needed cross-training to pursue integrative work linking across the genetic/molecular to cell to tissue scales.

PI: Larry A. Sklar
Cancer Research Facility Room 219A, MSC08-4630
University of New Mexico Health Sciences Center,
Albuquerque, NM 87131
T: 505-272-6892
lsklar@salud.unm.edu

PROJECT TITLE: 7 TMR (GPCR) Drug Discovery, Microfluidics & HT Flow Cytometry

PARTNERS:
Tione Buranda, PhD, Research Asst. Professor of Pathology, UNMHSC
Bruce Edwards, PhD, Research Professor of Pathology, UNMHSC
Gabriel Lopez, PhD, Associate Professor Chem. Engineering and Chemistry, UNM SOE
Tudor Oprea, MD, PhD, Professor Biochemistry and Molecular Biology, UNMHSC
Eric R. Prossnitz, PhD, Associate Professor of Cell Biology and Physiology, UNMHSC
Hy D. Tran, PhD, Asst. Prof. Mechanical and Electrical Engineering, UNM SOE
Andrea Mammoli, PhD, Asst. Professor Mechanical and Electrical Engineering, UNM SOE

GRANTING INSTITUTE: NIBIB.

ABSTRACT
High throughput (HT) screening is integral to drug discovery. While flow cytometry is known for its ability to measure cell responses, its power in the homogeneous analysis of ligand binding or molecular assembly and its potential for high throughput are not well-recognized. The possibility of displaying virtually any molecule in a format compatible with particle-based analysis as well as the novel approach of plug-flow flow cytometry for sampling times ~1 sec could make flow cytometry a powerful alternative for the real-time analysis of molecular interactions. Thus, we propose four projects that bring together expertise in bioengineering and biomaterials, receptors and cell biology, and flow cytometry instrumentation. The first two projects concern biomaterials. In the first project, we propose to express the proteins relevant to signal transduction and termination (seven trans-membrane receptors – 7TMR, receptor tails, G protein sub-units, arrestins, and receptor kinases) in forms appropriate for flow cytometry. These proteins will have epitope tags suitable for homogeneous attachment to beads as well as fluorescent groups suitable for detection by conventional flow cytometry. In the second project, we will employ biomaterial display and detection strategies compatible with flow cytometric analysis. Beads will be used as platforms to display the molecules, to analyze molecular assemblies, to examine enzymatic activities, and to examine inhibition by combinatorial drug libraries. Projects 3 and 4 will involve instrumentation development, fluidics, micro-machines, and automation. In the third project, we will develop fluid handling approaches for cells and beads. We will target throughput rates of 1 sample per second, or near the industrial standard of 100,000 samples per day, using commercial fluid handling components for the types of assays described in Projects 1 and 2. In the fourth project, we will develop and implement micro-fluidic sample handling approaches compatible with flow cytometry using novel elastomer-based micromachine technology. We have set a goal of 10 samples per second or 864,000 samples per day, exceeding the industrial throughput standard by nearly an order of magnitude. By integrating bioengineering, biomaterial, molecular, cellular and flow cytometric expertise, we expect to develop test platforms for high throughput analysis of molecular interactions with commercial potential in drug development. The resulting technological advances will allow us at the same time to define mechanistic details of cell activation through GPCR mediated pathways.

STATUS OF RESEARCH AND PARTNERSHIP

The UNM team has produced about 50 publications, manuscripts, inventions, and patents in its funding period. In the biological arena, we have established assays for high throughput flow cytometry that include: cell-based assays for GPCR and integrin ligand binding using fluorescent ligands; cell-based assays for GPCR initiated cell responses such as intracellular calcium elevation; cell-based adhesion assays for cells in suspensions; bead-based assays for GPCR molecular assemblies involving intracellular components; bead-based assays for GPCR tail peptide assemblies and phosphorylation; liposome/bead assays of transmembrane transport; and generalized bead-based approaches to analyze protein complexes. We have attacked a problem identified as key to drug discovery – HT single step discrimination of agonists, antagonists, and partial agonists for GPCR, the target of 50% of current prescription medicines. We have also developed a mathematical description of the molecular interactions that discriminate full and partial agonists and developed new approaches toward analyzing molecular interactions in signal transduction. In the technological arena, we have described instrumentation (Patent Pending, HyperCyt^{TM}) that approaches a rate of 100 samples/minute with <1% particle carryover from well to well. This approach uses air bubbles to separate □l sized samples with low carryover. We have developed a mathematical description of carryover. We have developed on-line microfluidic mixing strategies, including "wavy boundaries" for submicroliter samples and anticipate sampling rates up to 20 samples per minute. We have coupled HyperCyt^{TM} to high speed sorting and high speed multiplexing. We have also performed screens of chemical libraries in which computational and cheminformatic approaches have been integrated with HyperCyt^{TM}. Together, these approaches provide new opportunities for low cost, small volume, high throughput screening of cell and bead-based molecular target assays. As a result, the platform is now a tool for discovery of small molecules and probes, as well as characterization of molecular interactions and cell pathways. Furthermore, the platform is now enabling research into the discovery process itself. Thus, we are conducting experiments to understand how the combination of computational and physical screening can be used optimize the efficiency of discovery.

ISSUES

An important new part of the NIH roadmap is discovery research. One target of the discovery initiative is the development of molecular probes for biological systems including small molecules that can be used to investigate biological pathways. The roadmap has announced separate initiatives that create chemical libraries and chemical databases, develop high throughput screening assays and screening tools, and establish screening centers. Our BRP has anticipated and already begun to integrate these elements.

PI: Michael B. Smith, Ph.D.
Penn State College of Medicine
Center for NMR Research / Department of Radiology
500 University Drive
Hershey, PA 17112
T: 717-531-6069
F: 717-532-8486
mbsmith@psu.edu
http://cnmrr.hmc.psu.edu

PROJECT TITLE: High Field MRI: Limitations and Solutions

PARTNERS' NAMES AND AFFILIATIONS:
Kamil Ugurbil/Thomas Vaughan/Michael Garwood (University of Minnesota), Raymond Luebbbers (REMCOM), Steven Blackband (University of Florida), and Richard Briggs (University of Texas Science Health Center, Dallas)

GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB) and 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
In continuing the analysis of static magnetic (B0) field distortion artifacts (SA1) we focused our attention to the field distributions in the rats and mice, because of the rapid increase in research activities with animal models associated with molecular imaging and stem cell research. We have developed a whole body multi-tissue rat computer model. Using our developed 3D static magnetic field solver (d.1.1) we have evaluated Bo distributions in the rat model. The results are reported at 2004 ISMRM meeting. Utilizing these results (solver, digital animal model and Bo distribution), we are currently developing computer-guided passive-shimming methodologies and devices for animal studies (rats, mice and primates). The outcome of the animal study will be two fold: a) remove the critical obstacle of magnetic field inhomogeneity artifacts for animal MRI studies. These MRI studies are normally performed at high fields (4.7 T, 7.0 T or higher); b) gain experience for human experiments.

The proposed mGESEPI methods for artifact-free high T2* contrast and T2* mapping has been developed. We have implemented mGESEPI method on our 3T system and systematically carried out parameter optimization process with susceptibility phantoms, rat and human subjects. The clinical utilization of the mGESEPI method with on patients with micro-hemorrhage has been completed. A manuscript preparation reporting the results is underway. The clinical utilizations of the mGESEPI method have been demonstrated with initial studies on patients with Alzheimer's disease.

T2* measurement in normal human brain has been carried out with 14 normal subjects. The tissue iron in many brain structure strongly influence the T2* contrast in high field and has been implicated many neurodegenerative diseases. This model will provide foundation for quantitative evaluation of brain iron with T2* contrast in high field.

The proposed GESEPI-EPI method for dynamic artifact-free T2* imaging (fMRI, perfusion etc.) has been implemented on our 3T Bruker system. A significant development has been made in reduction of the magnetic field inhomogeneity artifacts by combining the GESEPI with SENSE encoding technology. The results have been presented at ISMRM, 2004 and a manuscript has been submitted to MRM.

Toward overcoming the RF magnetic (B1) field distortion problem, we have developed methodologies for phantom design that is based on the sample electrical characteristics in the presence of strong wave behavior. With this approach, the RF field and resultant image intensity distributions in the human body can be simulated more closely, compared we present an analysis of the electromagnetic wave inside biological samples, compared at different field strengths, and extrapolated to even higher field strengths. This method provides a useful tool for RF field engineering. The report summarizing this work is accepted by MRM.

In understanding the interaction between RF field and the human body in ultra high-field human MR imaging systems (7.0 – 8.0 T), we have carried out an experiment at 7.0 T and demonstrate that the RF field distribution in a human head can be drastically altered with water padding. The results provide experimental foundation that the RF field inside the human body at high fields can be effectively adjusted to a desirable distribution by proper placement of dielectric materials of certain geometries between the coil and sample; thereby passive shimming of the RF field can be performed. With increasing in RF field frequency up to 300 MHz at 7.0 T, this effect is considerably amplified. The report summarizing the results has been submitted to MRM, and is currently under revision.

ISSUES

The 32 CPU Beowulf cluster supercomputer was installed. Initially there were some difficulties with the hardware that have been resolved by replacement disk drives. Difficulties were encountered in obtaining the appropriate permission to perform experiments on cadaveric head at 11.7 T, however, they have been resolved and the experiments are scheduled for later this year.

PI: William A. Smith, D.Eng, P.E.
Department of Biomedical Engineering, The Cleveland Clinic Foundation
Dept. of Biomedical Engineering/ND-20, 9500 Euclid Avenue, Cleveland, Ohio 44195
T: (216) 445-9334
F: (216) 444-9198
wasmith@bme.ri.ccf.org

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

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

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 combined 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 increases both reliability and long-term durability. The "fail safe" controller will address the residual pinched wire, corroded solder joint, software hang-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 for VAD's, it is of a size both nationally and internationally to be of commercial significance. In the United States, it may exceed $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 preliminary in-vitro and in-vivo tests with the initial percutaneous system are completed. CCF developed a first version of an implantable wiring harness that was proven electrically reliable in two in-vivo studies. Future in-vitro and in-vivo test will serve to further evaluate and qualify the wiring harness. Under the CCF's guidance and the technical leadership of FMT, the actuator, implantable controller, implantable battery pack, transcutaneous energy transmission system and the external battery pack were successfully optimized, fabricated and integrated into the fully implantable TAH system. Three systems currently undergo extensive in-vitro and in-vivo studies while our partners fabricate and test components for two additional systems planned to join the in-vitro and in-vivo system tests in year 4. In addition, a battery endurance test stand and a bearing endurance test stand have been collecting valuable data since January 2004.

The partnership during year 3 worked successful on system integration overcoming difficult technical problems by communicating via telephone, email or design review meetings held at CCF or FMT. Extensive file sharing and referencing has guaranteed good program documentation that complies with FDA requirements.

ISSUES

Cost increases require careful consideration of program priorities to maximize the impact of the year 4 funding.

System integration was a major milestone in the program that enabled us to launch our in-vivo test program at the CCF. Communication among partners was important and proved a powerful tool to save time and push the program forward.

PI: Alan J. Snyder, Ph.D.
Departments of Surgery and Bioengineering
500 University Drive, C4864/H151
Hershey, PA 17033
T: 717-531-7068
asnyder@psu.edu

PROJECT TITLE: Biomedical applications of electroactive polymers

PARTNERS' NAMES AND AFFILIATIONS:
Qiming Zhang, PhD.
Materials Research Laboratory
The Pennsylvania State University
University Park, PA 16802

Mary I. Frecker, PhD.
Dept. of Mechanical Engineering
The Pennsylvania State University
University Park, PA 16802

GRANTING INSTITUTE: 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 work in the past year has focused on the development of new dielectric elastomers. This class of electroactive polymer is promising because of favorable mechanical characteristics, relative ease of analysis, and relative ease of processing. Most materials used to date are silicones or polyacrylate that provide high energy density due to their dielectric strength. We are developing high dielectric constant materials which we expect to provide similar energy densities at lower electric fields. These are being formulated as insulating polymer matrix-dielectric enhancer aggregates. Both two- and three-component systems have been studied, as have both plain aggregates and functionalized approaches where the enhancer is incorporated into the crosslinks of the matrix polymer.

The mechanical engineering partner has focused upon mechanical characterization of finished materials and development of both analytical and finite element models, also with an emphasis in this period on dielectric elastomers. 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 and processing requirements. Progress this year has included successful preparation of laminates capable of operating at higher pressures and demonstration of pumping at modest right heart pressures. We have also begun to study actuators that mimic hydrostatic animals.

ISSUES

The partnership is operating effectively. Whole-group meetings, one-on-one meetings and electronic communications among partners are all quite effective; we rely most upon electronic communications. Allowing graduate students from the different groups the freedom to consult and collaborate has been particularly helpful. Effective partners are motivated chiefly by the desire to work on new problems in a collaborative area. Joint funding enables them to devote the necessary time to the work.

PI: Evgeni Sokurenko, M.D., Ph.D.
University of Washington
Microbiology
357242
Seattle, WA 98195
T: 206-685 2162
F: 206-543 8297
evs@u.washington.edu

PARTNERS' NAMES AND AFFILIATIONS:
Viola Vogel (Institute for Biologically Oriented Materials, Swiss Federal Institute of Technology)
Wendy Thomas (Department of Bioengineering, University of Washington)
Ronald Stenkamp (Dept of Sructural Biology, University of Washington)

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

PROJECT TITLE: Dynamic Properties of Bacterial Adhesins

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

Prior to this year, we showed that the shear activation of bacterial binding was due to the ability of FimH to bind to mannose via "catch-bonds" that are longer lived when stretched by mechanical force. In this second year of our grant, our partnership has continued to demonstrate that nanotechnology and bioengineering tools can give enormous insight to problems in microbiology, and that biological components from microbiology can in turn create new nanotechnology tools.

We gained a much improved understanding of what types of bacterial adhesive behaviors are enabled by these catch-bonds. We demonstrated that *E. coli* display a "stick-and-roll" adhesion on mannosylated surfaces. This adhesion is counterintuitive; the bacteria roll along the surface in the direction of flow at moderate shear stress, but stop and stick firmly at high shear stress while they detach completely at low shear stress. All these modes are reversible if the shear stress changes. We also showed how some mutations in FimH and changes in the mannose structures affected this behavior. Together, these observations show a complex adhesive behavior with potentially profound implications for bacterial infection.

Toward understanding the structural basis for the FimH catch-bonds, we have demonstrated the importance of the non-adhesive "pilin" domain of FimH as a regulator of FimH behavior by testing the shear sensitivity of variants with mutations or deletions in this domain. This suggests that the minimal functional unit is at least as big as both FimH domains. We also developed a conceptual and mathematical model for a catch-bond that could explain the sticking and rolling as well as the earlier simulations and the importance of the pilin domain. (Previous catch-bond models could not). This model has been very helpful to the interpretation of the behavior of bacteria binding through the various FimH-mannose interactions, and should aid in quantitative nanotechnology engineering applications of catch-bonds.

Finally, we have shown a proof-of-principle for a nanotechnology application of FimH catch-bonds. There is currently no good experimental method for measuring shear stress in biological systems or other small aqueous systems such as blood vessels. However, we have shown that such a shear stress bio-sensor can be created using the cell-free system we developed last year with bacteria-derived purified fimbria and monomannosylated bovine serum albumin.

ISSUES

One of the technical issues that has arisen regards the atomic force microscope single molecule experiments. While nonspecific adhesion of proteins to plastic has worked well for flow chamber assays, it has not worked well for these experiments, and we are currently looking for ways to bind the fimbriae or a stabilized FimH construct to the surface.

A related technical issue has been in incorporating cystein residues into FimH in order to attach it to surfaces or to add fluorescent markers to specific sites. We think the cysteins sometimes interrupt the normal disulfide bonding in the protein.

The partnership is healthy and growing. Viola Vogel, the bioengineer in the partnership, is moving to ETH in Zürich. We anticipate that the move to Zurich will expose us to new colleagues with new techniques and insight. In addition, we have added Wendy Thomas, who will be an assistant professor of Bioengineering at the University of Washington next year, as an additional partner to the grant.

PI: Steven A. Soper
Department of Chemistry
232 Choppin Hall
Louisiana State University
Baton Rouge, LA 70803-1804
T: (225) 578-1527
F: (225) 578-3458
chsope@lsu.edu

PROJECT TITLE: Micro-Instrument Platforms for Genetic-Based Assays

PARTNERS' NAMES AND AFFILIATIONS: Robin L. McCarley (Chemistry, LSU); Robert P. Hammer (Chemistry, LSU); Michael C. Murphy (Mechanical Engineering, LSU); Wanjun Wang (Mechanical Engineering, LSU); Dimistris Nikitipoulos (Mechanical Engineering, LSU); Kevin Kelly (Mechanical Engineering, LSU); Jost Goettert (CAMD, LSU); Francis Barany (Molecular Genetics, Cornell Medical College).

GRANTING NIH INSTITUTE/CENTER: National Institute of BioImaging and Biotechnology (NIBIB)

ABSTRACT
The goal of this research effort is to bring together a multi-disciplinary team (Chemists, Engineers, Life Scientists) to develop integrated microfabricated tools to carry out PCR/LDR (Polymerase Chain Reaction/Ligase Detection Reaction) assays for the detection of low abundant diagnostic markers (K-ras mutations, 19 single base mutations) specifically for colorectal cancers. The devices to be fabricated as part of this research program are:

- Fast thermal cyclers for PCR amplification of DNAs in nanoliter volume chambers with automated sample and reagent delivery.
- Micro-electrophoresis devices machined in polymethylmethacrylate (PMMA) containing multiple separation channels for the high speed processing of ligation products.
- DNA hybridization chips (micro-arrays) fabricated in plastics with nano-fluidic channels for LDR capture. The DNA micro-array substrates will offer robust immobilization chemistries that can tolerate typical thermal and chemical DNA hybridization/denaturation conditions.
- Ultrasensitive near-IR fluorescence instrumentation using solid-state components (diode lasers and detectors), which can be operated in a scanning mode to read multiple electrophoresis channels and/or DNA micro-arrays with extremely high sensitivity.
- Ultra-bright near-IR fluorescent probes appropriate for use with miniaturized near-IR detectors and for DNA micro-array readout. These probes will be configured in a two-color, four-lifetime format, which will allow the simultaneous readout of **8** unique probes.
- Injection molding of miniaturized plastic devices for maximizing production rates and minimizing fabrication costs.
- Materials characterization of the unique substrates that will be used for the micro-electrophoresis and hybridization-based assays. Also, new bonding procedures will be investigated to aid in device assembly.
- Micro-pumps for nanoliter per minute volume flow rates in microchannels used for fluid handling in pressure driven systems.

STATUS OF RESEARCH AND PARTNERSHIP

We are starting the fifth year of our project and are formulating plans for a competitive renewal of our BRP application. All of the original participants of our BRP (see above) are still actively involved in this project and are anticipating contributing to the competitive renewal. In the renewal, we will have a more active participation of our collaborators at Cornell Medical College (Barany) and also, bringing in another collaborator, Dr. Pat Paty from Sloane Kettering Memorial Cancer Institute, also in New York. Our renewal will focus on fabricating new microfludic devices for the following two general areas associated with the clinical diagnostics of colorectal cancers:

- Molecular profiling of solid tumors (to provide personalized treatment).
- Molecular profiling of shed epithelial tumor cells found in circulating peripheral blood (to provide early detection).

A number of new assays have been formulated by our BRP team to take advantage of our unique capabilities in terms of device fabrication, new fluorescent probe developments and single molecule detection to allow new paradigms in molecular diagnostics that approach real time reporting. These capabilities were a direct result of our current BRP application. Most of our existing devices were fabricated in single channel formats and will be scaled up to 96-channel formats to accept input directly from microtiter plates. We have developed techniques to allow large area replication of polymer parts with extremely high aspect ratios and exquisite lateral dimensions. These polymer parts are inexpensive and simple to fabricate, making them particularly attractive for diagnostic applications where cross-contamination can result in false positives. Therefore, these clinical applications demand disposable-type formats without scarifying high-throughput capabilities.

Our current BRP has produced the following results:

- Over 40 peer-reviewed publications in a four year period of time have resulted from our BRP initiatives, which are joint publications between various members of the research team.
- New multi-investigator efforts have evolved from the formation of this BRP team. For example, we have received funding from the National Cancer Institute through their R21/R33 Innovative Molecular Analysis Technology (IMAT) program to develop devices for selectively capturing low abundant cells in peripheral blood. In addition, we have just received an infrastructure development award from the National Science Foundation to spawn a National Center of Excellence focused on developing new materials and micro- and nanosystems for applications in a variety of areas. Louisiana State University has made a commitment of space (33,192 sq. ft. building) to house this Center and the extensive inventory of state-of-the-art equipment to support microfabrication and also, develop capabilities in nanofabrication.
- Our BRP has supported a number of different researchers over the four-year funding period and has allowed the training of students receiving terminal degrees (Ph.D., M.S.) in engineering and chemistry. We have supported five post-doctoral associates (2 currently), nine Master students (4 currently), eight Ph.D. candidates (4 currently), two research assistants (1 currently), and three undergraduates (1 currently). Our Ph.D. graduates are currently doing research at government (Oak Ridge National Laboratories, National Institute of Standards and Technology), private (TransGenomics) and academic laboratories (University of Alabama-Huntsville, Mississippi State University).

ISSUES

There are no technical or logistical issues associated with our BRP project at the present time. However, since our group was one of the original grantees under this funding mechanism in 1999, it would be helpful to hear information on what constitutes a successful BRP effort to warrant successful funding from a competitive renewal. Due to the new nature of this funding mechanism and the fact that it is different from a traditional R01 mechanism, it seems as if the review criteria for BRP efforts may be substantially different from competitive renewals through R01 mechanisms.

PI: Patricia A. Thiel
Iowa State University
Ames, IA, 50011
T: 515-294-7871
F: 515-294-4709
Thiel@ameslab.gov
http://www.public.iastate.edu/~pthiel/

CO-PI: Svetlana A. Shabalovskaya
Iowa State University
R324 Wilhelm Hall
Ames, Iowa, 50011
T: 515-294-1293
Shabalov@ameslab.gov

PROJECT TITLE: Design of Biocompatible NiTi surfaces

PARTNERS' NAMES AND AFFILIATIONS
Iowa State University: James Anderegg, M.S., Joan Cunnick, Ph.D.
Institute of Energy and Interface, CNR, Italy: Gianni Rondelli, Ph.D.
University of Washington: Kip Hauch, Ph. D.
Medical College of Georgia School of Dentistry: John Wataha, Ph.D.

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

ABSTRACT
Because of the thin profile of miniature shape memory and superelastic medical devices and better performance of bare Nitinol (a group of nearly equiatomic Ni-Ti alloys) stents, native biocompatible surfaces are of interest for medical applications. This proposal brought together a multi-disciplinary team of investigators to focus on the design of Nitinol surfaces for long-term implantation. Eight different Nitinol surfaces (versus 1-2 customarily employed) were explored and tested in corrosion and biological studies in this project. The following conclusions are drown from the performed surface studies: Despite a general tendency to form Ti-based surface oxides the presence of Ti and Ni in equal proportions in the alloy as well as a strong gradient in Ni concentration in the surface sub-layers provide conditions for the modification of NiTi surface chemistry in a wide interval of Ni and Ti concentrations. Processing of the material that results in alteration in the alloy composition due to the formation of intermetallic precipitates at high temperatures, Nitinol melting practices resulting in inclusions (oxides, carbides, and carbonates), the regimes of electrochemical treatments and chemical solutions are the factors that must be controlled to design homogeneous, stable, and biocompatible Nitinol surfaces.

STATUS OF RESEARCH AND PARTNERSHIP
Surface chemistry. Amorphous state was the most common state of Nitinol surfaces prepared in this study. It was induced either because of the high degree of disorder caused by plastic deformation during surface preparation or selective leaching of Ni upon exposure to chemical solutions. Surface crystallization was induced only at T~ 700^{o}C that was far above the temperatures used for Nitinol shape setting. The amorphous state of Nitinol surface oxides may benefit the corrosion performance of Nitinol, provide flexibility matching that of the bulk of the alloy, and, by means of inhibition of fibrinogen denaturation, may reduce propensity for thrombosis. Nitinol surfaces revealed two types of chemical heterogeneity associated with elemental or oxide segregation (Ni-based vs Ti-based oxides) induced by slight heating of plastically deformed surfaces and with the presence of inclusions (Ti oxides, complex TiNi-based oxicarbides and carbonates). The existence of inclusions in the bulk of Nitinol is common

knowledge but their nature, origin, distribution, effect on corrosion resistance, and biological performance had never been explored before. Depending on the type of treatments (polishing, electropolishing, chemical etching, heat treatment, etc) Ni surface concentrations varied from zero to 8 at.%, and those of Ti from 6 % to 23%, providing Ti/Ni ratios in the range from 2 to 28.

Ni release and corrosion studies. Ni concentrations in the biological medium exposed to the designed surfaces varied from 0 to 11 ng/ml. This is in the range of the natural level of Ni in human serum. Very low Ni release is assigned to the formation of protein coating on the surface, inhibiting ion exchange on the interface. Surprisingly, the major concern has not been Ni release but the presence of particulates (inclusions) on Nitinol surfaces. Preliminary corrosion studies showed that the Nitinol corrosion resistance could be tremendously improved if the surface were relieved of these inclusions. The presence of inclusions of varied chemical composition on the surface can also contribute to the low scratch healing ability of Nitinol. Accumulation of particulate material around Nitinol implants with inclusions on the surface is inevitable with time. Surface chemical heterogeneity also affects biological performance through protein adsorption inhibiting uniform cell coverage. Corrosion resistance of electropolished surfaces proved to be inferior to that of chemically etched ones because electropolishing smoothening the surface leaves, however, all inclusions behind. Comparing the corrosion performance of several Nitinol wires for medical use, we concluded that sand blasting is not an efficient procedure for the removal of the original black oxide from the wire. Sand blasting occasionally leaves original scale and causes fragmentation of inclusions on the surface contributing in the inconsistent corrosion performance. Inclusion fragments may be imbedded into the surface layers during final drawing of sand blasted wire causing higher inclusion concentration on the final stage of wire processing.

Biological studies (one trial of each study). Five biological studies in human model were performed to assess the biological performance of Nitinol. The toxic response was evaluated using peripheral blood lymphocytes (PBL) and endothelial cells (HVEC). In the studies of albumin vs fibrinogen adsorption and platelet responses, the propensity for platelet-induced thrombosis was explored. Nitinol surfaces with different morphology that induced nanoscale Ni release caused very low, if any toxic effect on EC. A 15-30% stimulation of human PBL was observed similarly to an earlier study on Lewis rats. The biological effects induced by subtoxic Ni concentrations must be considered based on Ni essentiality, rather than its toxicity. Protein adsorption and platelet responses were strongly dependent on the surface chemistry. Fibrinogen adsorption varied in direct proportion to the Ti surface concentration and albumin revealed affinity to Ni-containing surfaces. Nitinol surfaces that adsorbed more albumin showed lower platelet coverage. The tendency towards platelet aggregation in thrombus-like structures depended on surface treatment of Nitinol. A new avenue to explore in search of Nitinol surfaces with better thromboresistance would be the Ni-containing Nitinol surfaces that induces very low Ni release, rather than surfaces built exclusively of Ti oxides.

ISSUES

Despite a tight budget, extremely intense research plan, unexpected difficulties in obtaining material, hiring a postdoc, etc, and increased volume of work compared to that originally planed, our international team of sprinters (American, Russian, Italian, and a Chinese postdoc) did very well during this two year project that was extended to third year to finish important biological studies, analyze all data, and present them to public. Six publications were generated and most of them have been already published. We are ready to enter a second round - a five year program generated based on the pilot studies conducted in the first part of the project. Thank you very much for the interest in our work and funding from the first submission.

PI: Antoni P. Tomsia, Ph.D.
Materials Sciences Division
Lawrence Berkeley National Laboratory
MSD Building 62R0203
Berkeley, CA 94720
T: 510-486-4918
F: 510-486-4761
aptomsia@lbl.gov

PROJECT TITLE: Complex Nanocomposites for Bone Regeneration

PARTNERS' NAMES AND AFFILIATIONS:
Materials Sciences Division, Lawrence Berkeley National Laboratory, University of California, Berkeley; Departments of Chemistry and Molecular and Cell Biology, University of California, Berkeley; Department of Materials Science, University of California, Berkeley; Department of Preventive and Restorative Dental Sciences, University of California, San Francisco.

GRANTING NIH INSTITUTE/CENTER: 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. The ultimate goal is to develop strong and tough implant materials for load-bearing applications deriving their strength from nanoparticle hydroxyapatite and their toughness from hydrogel polymers, with the microstructural architecture scale on the order of tens of nanometers and below. Three types of materials will be developed. First, inorganic scaffolds with a dense core and a *graded distribution of porosity* and surface chemistry will be fabricated by stereolithography and by a novel technology developed in our laboratory based on freeze casting of calcium phosphate suspensions. Second, hydrogels and self-assembling polymers that possess anionic groups and adhesive ligands suitably positioned for the nucleation process and cellular adhesion will be used to direct template-driven biomimetic mineralization of hydroxyapatite and other biominerals in nanoscopically and microscopically controlled fashion. Third, the resultant porous scaffolds will be used as the matrices to fabricate inorganic-organic composites with improved strength and fracture resistance. This will be achieved by infiltration of the inorganic scaffolds with hydrogels or by direct template-driven biomimetic mineralization of calcium phosphate nanoparticles on the organic scaffolds. Materials that pass the mechanical property tests will be tested in cell cultures and an animal model.

STATUS OF RESEARCH AND PARTNERSHIP
The first year of the project has been very productive with significant progress having been made on most of the specific aims. We have initiated work centered on the fabrication of organic-inorganic composites for bone replacement using two basic approaches: infiltration of porous inorganic scaffolds with different polymers, and mineralization of organic scaffolds. During the first year we focused on three areas: (1) we developed new techniques for the preparation of porous inorganic materials; (2) we discovered novel method for the mineralization of polymer scaffolds, and, (3) because optimum bone replacement materials will require control of the interactions between the bone cells and the materials surface, we also began research in this direction.

1. Preparation of porous inorganic scaffolds. In order to determine the optimum conditions for the sintering of porous calcium phosphate scaffolds with controlled density and particle size were determine, we have carried out a systematic study to analyze the densification and grain growth of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP). Preliminary studies examined freeze casting of HA as a technique for the fabrication of porous complex-shaped ceramic scaffolds. We have established the adequate casting conditions for the preparation of porous scaffolds with an anisotropic microstructure consisting of parallel interconnected open channels separated by ceramic plates aligned along the ice growth direction. On the internal walls of the channels, a dendritic, branch-like structure could be observed following the microscopic ice formation. Depending on the speed of the freezing front and the solids concentration in the initial slurries, the sintered scaffolds had relative densities between 30 and 70% and the width of the channels typically ranged between 50 and 200 μm.

2. Mineralization of polymer scaffolds: Urea-mediated mineralization of pHEMA-based hydrogel copolymer scaffolds. We have developed a novel mineralization approach for integrating HA with poly(2-hydroxyethyl methacrylate)-based (pHEMA-based) hydrogel copolymers with strong polymer-mineral interfacial adhesion strength. This mineralization method takes advantage of the dramatically different solubilities of HA in acidic and basic aqueous solutions and the chemically labile nature of the ester groups of pHEMA in basic solutions. By generating in situ surface carboxylates, strong binders for calcium ions, this process provides a fast and convenient approach for producing robust pHEMA-calcium apatite composite materials with high-quality interfacial integration between the mineral and the polymer substrate. By designing the appropriate surface chemistry, the adhesion of the organic and inorganic phases can be modulated, resulting in materials with improved mechanical properties. The technique could be extended to mineralization with other inorganic phases and could be used for the fabrication of bulk organic-inorganic materials.

3. **In vitro evaluation of interactions between bone cells and mineral-binding ligands via two-dimensional functional surfaces**. Controlling the interactions between bone cells and materials surfaces is required in order to design materials with improved osseointegration. We propose the use of 2-D functional surfaces to assist in the evaluation of interactions between bone cells and mineral-binding ligands used in the construction of 3-D biomimetic hydrogel scaffolds. To realize this strategy, we first developed a surface functionalization approach based on plasma treatment and straightforward bioconjugation chemistry. Specifically, primary amine-functionalized glass slides obtained through a multi-step plasma treatment were conjugated with anionic amino acids that are frequently found as mineral-binding elements in acidic extracellular matrix components of natural bone. The modified glass surfaces were characterized by X-ray photoelectron spectroscopy (XPS) and contact angle measurements. Human osteosarcoma TE85 cells were cultured on these functionalized slides and analyses on both protein and gene expression levels were performed to probe the "biocompatibility" of the modified surfaces. Cell attachment, proliferation, and the expression of the cytoskeletal protein α-tubulin were comparable to those of cells cultured on tissue culture polystyrene (TCPS). The modified glass surfaces promoted the expression of osteocalcin, alkaline phosphatase activity, and ECM proteins such as fibronectin and vitronectin under differentiation culture conditions. Transcript analysis using gene chip technology confirmed that culturing TE85 cells on anionic surfaces did not activate apoptotic pathways. Collectively, these results suggest that the potential mineral-binding anionic ligands examined here do not exert significant adverse effects on the expression of important osteogenic markers. Our results pave the way for the incorporation of anionic ligands into biocompatible three-dimensional artificial bone-like scaffolds and will help to increase our understanding of the body response to bone replacement materials.

ISSUES
None

PI: Robert T. Tranquillo
Professor and Head
Department of Biomedical Engineering
University of Minnesota
7-114 BSBE
312 Church St SE
Minneapolis, MN 55455
T: 612-625-6868
tranquillo@cems.umn.edu

PROJECT TITLE: Tissue-engineered Valve from Cell-Remodeled Biopolymer

PARTNERS' NAMES AND AFFILIATIONS:
(all at the University of Minnesota except as noted)
Victor Barocas, Ph.D., Department of Biomedical Engineering
Emad Ebbini, Ph.D, Department of Electrical and Computer Engineering
Mark Nicosia, Ph.D, Exponent Consulting
Sarah Shumway, M.D., Department of Surgery
Catherine Verfaillie, M.D., Department of Medicine and Stem Cell Institute

GRANTING NIH INSTITUTE/CENTER: 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, will be used. A tissue mechanical theory will be applied to determine the optimal mold design such that cell-mediated compaction of the gel around the mold surfaces yields the target geometry and ECM fiber alignment. A coupled solid-fluid mechanical model of valve function in pulsatile flow will be used to define what alignment-dependent mechanical properties of our "valve-equivalent" (VE) are desired following incubation for proper valve function, and to simulate what the VE function will be. Various experimental strategies will be implemented to manipulate these properties during incubation. Measurements of these properties will be used to develop a microstructural constitutive model of the tissue resulting from the cell-remodeled gel that is needed in the model of valve function. High-speed ultrasonic imaging of leaflet motion will be developed and used along with particle imaging velicometry in order to validate the model as well as visualize valve function. In addition to comprehensive biological and biomechanical characterization of the VE, novel adult stem cells will be assessed as a source of endothelial cells and, potentially, interstitial leaflet cells for VE fabrication. An animal study will be performed to access the remodeling that occurs in vivo and its effect on valve function.

STATUS OF RESEARCH AND PARTNERSHIP

This BRP has entered into its second funding year. The project was fully staffed by the end of the first year. Progress is being made as expected on all Specific Aims, with several projects approaching the point of initial publications.

Specifically, a method and has been developed for casting fibrin-based valve constructs ("valve-equivalents", or VEs) and effort is being focused on attaining the desired fibrin remodeling into cell-produced tissue with requisite functional properties. Experiments are ongoing comparing fibrin remodeling among candidate cell sources (fibroblasts, valve interstitial cells, and cells differentiated from adult stem cells). A 3D FEM simulation code to predict contraction of the fibrin gel (induced by the entrapped cells) in the mold is running and will guide mold modifications to achieve the desired circumferential alignment in the root and commissural alignment in the leaflets. A prototype bioreactor to impart "flow conditioning" on the VE as the tissue develops in vitro is being characterized. A novel computational method for solving the "fluid-structure interaction" (FSI) problem in valve function is developed to the point of solving the steady flow problem in an idealized 2D problem. 3D particle imaging velocimetry (PIV) equipment has been acquired and validated for unconfined flows and is currently being calibrated and validated for confined flows relevant to valve function. The speckle characteristics of pulse-echo ultrasound (US) data from VEs have been investigated and the ability to image the VE leaflets using a 9 MHz Small Parts Probe and a 20 MHz Focused Transducer has been established. A novel 2D speckle tracking method has been developed for high-resolution displacement/strain measurement for the VE tissue. The FIS, PIV, and US work is being pursued in coordination with the bioreactor development for obvious reasons.

There have been some changes in the identities and roles of the investigators due to departures from the University of Minnesota: Ted Oegema is now at Rush Medical Center and considered a consultant for tissue biochemistry matters; Mark Nicosia is now at a consulting firm and will likely continue his involvement, although at a reduced level, with Victor Barocas assuming the primary role for the PIV and FSI work.

ISSUES

Besides the usual issue of getting a new project of this scale fully staffed, the only issue has been developing a contingency plan for the status change of the investigators noted above. A likely reason why there are no other issues to report is because for the first year, all of the investigators (besides Ted Oegema, who moved soon after the grant began) were at the University of Minnesota. This greatly facilitates the interactions needed for this project to succeed.

PI: Philip Troyk, Ph.D.
Illinois Institute of Technology
Pritzker Institute of Biomedical Science and Engineering
IIT Center 10 W 32nd St, E1-116
Chicago, IL 60616
T: 312-567-5324
F: 312-567-5707
troyk@iit.edu
http://neural.iit.edu/intro.html

PROJECT TITLE: Intracortical Visual Prosthesis

PARTNERS' NAMES AND AFFILIATIONS:
Philip Troyk (Illinois Institute of Technology), David Bradley (University of Chicago), Stuart Cogan (EIC Laboratories, Inc.), Robert Erickson (University of Chicago), Doug McCreery (Huntington Medical Research Institute), Vernon Towle (University of Chicago) (space does not permit listing all partners)

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?

The overall objectives of the Intracortical Visual Prosthesis BRP are to advance the technology sufficiently to provide a reasonable expectation of reliability and safety for implantable hardware, and to develop an animal model to perform crucial psychophysical and electrical stimulation studies. This 4-year project will culminate in an analysis of data from the fundamental electrical stimulation and psychophysical studies of an animal model, and the development of a completely implantable multichannel stimulation system, including chronically implantable stimulation electrodes. Our long-term goal is to develop an implantable system that will provide usable vision for a large population of persons with blindness. The goals of this 4-year project are to answer the fundamental questions, above. Our short-term goals are to: 1. Develop a primate animal model for testing the sensory responses to large numbers of parallel intracortical stimulation electrodes. 2. Extend earlier human work on point-phosphene

perception to a more general approach that tries to exploit other V1 tuning properties, such as orientation selectivity, to create a richer visual feature set. This will be done by a combination of recording and stimulation techniques in highly trained monkeys performing psychophysical tasks. 3. Demonstrate safety, efficacy, and electrochemical stability of our proposed intracortical electrode arrays using a combination of in vitro and in vivo testing. 4. Determine the optimal physical configuration for, and design a high-reliability implantable inductively-powered cortical stimulator, interfaced to an external computer controller. 5. Develop safe implantation methods, including pre- and postoperative imaging techniques, to optimize and minimize the duration of implant surgical procedures.

STATUS OF RESEARCH AND PARTNERSHIP
Progress has been made in all three of the critical areas.

Physiology: 3 non-human primates have been implanted with 100-200 electrodes per animal and psychophysical studies have validated the feasibility of using an animal model to explore visual prosthesis functionality, stimulation coding strategies, and surgical placement of electrodes.

Electrode Technology: An unprecedented database of cyclic voltammagrams, pulsed electrode waveforms, and photographic characterization of over 400 electrodes has been assembled. Analysis of the data has revealed reliability issues common to all intracortical electrode designs. A more thorough understanding of activated iridium oxide and how to safety use it to inject charge has emerged.

Implantable hardware: We have come to realize that use of cables to bring stimulation signals to subdura arrays of electrodes is problematical. Therefore we have designed a wireless array/module that can service 16 electrodes. The wireless design allows for no wires to cross the dura, enhancing the surgical feasibility of implanting ~1000 electrodes in area V1.

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

ISSUES
Presently there are two key issues that our team is agressively devising studies to address: Electrode design and consistency, and the use of our animal model to demonstrate the feasibility of integrating multiple artificial visual percepts.

PI: Akira Tsuda, Ph.D.
Physiology Program
Harvard School of Public Health
Environmental Health
665 Huntington Ave
Boston, MA 02115
T: 617-432-0127
F: 617-432-3468
atsuda@hsph.harvard.edu

PROJECT TITLE: Particles in the Developing Lung: Bioengineering Approach

PARTNERS' NAMES AND AFFILIATIONS:
Wolfgang Kreyling, Holger Schulz, Manuela Semmler, Claudia Reinhard and Joachim Heyder (GSF-National Research Center for Environment and Health, Munich, Germany), Peter E. Hydon and Fiona Laine-Pearson (Dept. of Mathematics and Statistics, University of Surrey, U.K.)

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 newborn, children, and adult lungs have not been addressed in 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 the postnatally developing rat animal model. These proposed studies will allow us, for the first time, to get a comprehensive picture of 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 first year of this BRP project, research is progressing as planned. Analytical investigation: To provide theoretical underpinning of how the parameters governing the geometry and flow in the alveolus determine the extent of chaotic mixing, we are currently expressing the equations of fluid particle motion in the moving-walled model alveolus as an integrable Hamiltonian system with a perturbation, which depends on the Womersley number (□), the Reynolds number (*Re*), and the wall deviation parameter (□). We study how these parameters govern the extent of Hamiltonian chaos in this model. Computational investigation: We have started to develop a finite element computational model of a rhythmically expanding/contracting alveolated duct to study the potential effects of developmental changes in acinar architecture on alveolar wall motion. The rationale for this modeling is that asynchronous motion of acinar airway architecture is known to cause acinar flow mixing and consequently enhance aerosol particle mixing and deposition. The initial results indicate that differences in the hysteretic behavior of surfactant and ductal ring tissue may contribute to acinar geometric hysteresis and therefore to asynchronous flows and enhanced aerosol deposition deep in the lung.

On the experimental side, we are working in two areas. (1) We are developing a state-of-the-art inhalation apparatus and particle clearance/retention detection system for postnatally developing rats. The initial performance test showed that with this system 7, 14 and 21 days old rats can be exposed to Ir-192 radio-labeled iridium aerosols (18 – 22 nm count median diameter generated at $1\text{-}10\text{x}10^{6}$ cm^{-3} concentrations by spark ignition) for 1 hour at 120 breaths per minute at a tidal volume of about 80% of total lung capacity. (2) We are also developing a PC-control high precision lung function test unit for postnatally developing rats. The initial performance test of this showed that the minimized instrumental deal space is small enough to provide adequate resolutions to perform lung function studies in adolescent rats aged 35 or 90 days.

The first BRP partnership meeting was held at Munich (GSF site) on March 29-31. The project leaders of all sites and all investigators/staff members at the GSF site attended this meeting. We discussed the overall research objectives, five-year long term research plan, current year research plan, as well as administrative/programmatic research logistics. The meeting was mutually beneficial and highly productive. The second mini BRP partnership meeting was held at the Harvard site on May 20, 2004. The PI (Dr. Tsuda) and the project leader of the GSF site (Dr. Kreyling) exchanged the updated information on research progress of each site.

The PI is currently in the process of establishing an additional partnership with the Center of Supercomputing at the University of Kragujevac, Serbia (the project leader: Dr. Vlastelica). The Center of Supercomputing at the Univ. of Kragujevac is well known in its expertise for computational modeling of the mechanical coupling between fluid dynamics and solid (material) mechanics and this site will develop a state-of-the-art finite element computational model of acinar architecture at different stages of postnatal lung development.

ISSUES: None, except initial administrative delay in establishing foreign subcontracts.

PI: Geoffrey D. Vince, Ph.D.
The Cleveland Clinic Foundation
Department of Biomedical Engineering
9500 Euclid Avenue
Cleveland, OH 44195
T: 216-444-1211
vince@bme.ri.ccf.org

PROJECT TITLE: High Frequency Nonlinear Acoustic Intravascular Imaging

PARTNER'S NAMES AND AFFILIATIONS:
Aaron Fleischman, PhD; Shuvo Roy, PhD; Nikolay Kharin, PhD (all BME,CCF);
Murat Tuzcu, MD; James Thomas, MD (all Cardiology, CCF); Cheri Deng, PhD (BME at Case Western Reserve University); Dov Hazony, PhD (Electrical Engineering & Computer Sciences at Case Western Reserve University); Lawrence Katz, PhD (BME at University of Missouri – Kansas City)

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

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

STATUS OF RESEARCH AND PARTNERSHIP:
We continued our work on modeling of nonlinear propagation in blood and similar blood-like nonlinear organic fluids (Specific Aim 1). In diagnostic ultrasound (US) the nonlinear effects and effects of absorption are often comparable and US does not produce shock or even strongly distorted waves. This allowed for the development of the set of novel equations to describe the behavior of the US wave in blood, other body fluids and tissue. Our latest results on moderately nonlinear ultrasound propagation in blood-mimicking fluid were published recently. We are planning future experiments to investigate the nonlinear US propagation through the tissue layers and the role of variable absorption in them. The major breakthrough in experimental verification of the theory, developed pursuing Specific Aim 1, occurred when we obtained an acoustic probe with the spot size of 0.04 mm, which is about one wavelength at frequency 40 MHz. This is a needle broadband hydrophone (Precision Acoustics Ltd., UK). We originally proposed to characterize our transducers, developed pursuing the Specific Aim 2, using a hydrophone because the measurements of the beam patterns in the free field produce the most reliable data. However, due to a lack of such a precise probe we made a number of experiments with imaging phantoms, which provided the basic information on these high frequency transducers parameters. Specific Aim 2 is still our main priority. Our custom-engineered PVDF transducers provide 110 -115 % bandwidth with second harmonic level - 25 to - 20 dB. At the same time traditional piezoceramic transducers, probably, are able to generate second harmonic signal more effectively, however, they are not broadband enough for effective reception of the second harmonic pulse-echo.

ISSUES:
None.

PI: Tuan Vo-Dinh
Oak Ridge National Laboratory
P.O. Box 2008
Oak Ridge, TN 37831-6101
T: 865-574 6249
vodinht@ornl.gov

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

PARTNERS:
Thompson Cancer Survival Center, Knoxville, TN 37916 (M.Panjehpour and B.F. Overholt) College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37916 (R. DeNovo)

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

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

Laser-induced fluorescence (LIF) spectroscopy has already been used to detect cancer and high-grade dysplasia in Barrett's esophagus. However, that system uses a contact technique, which samples a 1 mm area of tissue at each measurement. While the contact LIF system is better than the pinch biopsy technique, a new system is needed to allow examination of the entire surface of the mucosa. To address this important need in imaging, we will develop a real time synchronous imaging system based on state-of-the-art acousto-optic tunable filter (AOTF) technology 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 important progress in several aspects of the project. We have completed the design, construction, and initial testing of a novel intra-operative multi-modality multispectral imaging (M^2-MSI) system. This M^2-MSI system provides the unique capability to simultaneously perform refection imaging using white light and fluorescence imaging of tissue using the same tunable filter platform. The M^2-MSI system employs laser excitation, an imaging fiber probe system for signal collection, endoscope, an acousto-optic tunable filter (AOTF) for wavelength selection, a

charge-coupled device (CCD) for refection detection and an intensified charge-coupled device (ICCD) for fluorescence detection. Previously, we successfully imaged tumors in laboratory mice that had been injected with an exogenous fluorophore (e.g., porphyrin) using an earlier version of the MSI system. The new MSI system was equipped with a tunable optical parametric oscillator (OPO) laser; hence we were capable of tuning our excitation light over the spectral range of 405 - 700 nm.

The phase of this project involved the development of a dual-mode imaging system that is capable of acquiring real-time fluorescence and reflection images. Whole tissue was imaged with a fixed-wavelength excitation at 337 nm using a 3-mW pulsed nitrogen laser with a maximum repetition rate of 20 Hz and a 512x512-pixel ICCD camera. Reflection images were acquired using an Olympus endoscope illuminator. In addition to the imaging capability, we successfully coupled an additional device for spectroscopic measurements thus enabling us to perform multimodal hyperspectral imaging. In addition, single-point measurements from areas of normal and sites of malignant tissue were conducted simultaneously with the imaging system. Using the multimodal MSI system, we have successfully performed spectral imaging measurements to monitor quantitatively and qualitatively differences in the naturally occurring cellular fluorophores in tumor and normal mice tissues provided by Dr Steve J Kennel at ORNL. We are working closely with our partners, the Thompson Cancer Survival Center (TCSC) and the University of Tennessee-Knoxville (UTK) on this project. We have performed evaluation tests of our instrumental systems using canines at the UTK College of Veterinary Medicine. The protocol for clinical studies has also been approved by the Internal Review Boards (IRBs) at both institutions (TCSC, ORNL).

In addition to the MSI work, we have continued to conduct single-point measurements using our laser-induced fluorescence (LIF) system. Previous measurements were conducted to optimize the time gate and delay parameters of the LIF spectral device in order to obtain maximum signals with minimum interference from the laser scatter. Following these optimization procedures, we have improved the device from a single-wavelength excitation to multispectral point-measurements using a nitrogen-dye laser for excitation. This instrumental setup has several advantages over our previous fixed-wavelength excitation LIF system since the new system incorporates the capability for multiple excitation wavelengths and the possibility for time-resolved measurements using an ICCD. Currently clinical studies are being performed using the endoscopic time-resolved LIF measurements at various excitation wavelengths ranging from 400-480 nm on human patients.

ISSUES

N/A. We have completed the MSI prototype which will be used for clinical testing.

PI: Alan Waggoner, Ph.D.
Carnegie Mellon University
Molecular Biosensor and Imaging Center
4400 Fifth Avenue
Pittsburgh, PA 15213-2683
T: 412-268-3456
F: 412-268-6571
waggoner@andrew.cmu.edu
http://www.cmu.edu/bio/faculty/waggoner.html

PROJECT TITLE: Long Wavelength Quantum Dot-based probes – cell tracking

PARTNERS' NAMES AND AFFILIATIONS:
Marcel Bruchez (Quantum Dot Corporation)

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

ABSTRACT
We are developing new technologies to employ quantum dots in biological imaging in vitro. In an evolving program, quantum dot technology will be developed specifically for (1) cell identification and tracking of cells in tissues, (2) tracking cell proliferation through multiple generations in tissues (3) vasculature location, and (4) deep 3-D imaging of Qdot-labeled polymer scaffold structures relative to the Qdot-labeled cells in engineered tissue models. Quantum Dots Corporation will prepare new types of Qdots that have properties suited to deep imaging of cells and structures in tissues. Particular emphasis will be placed on near infrared Qdots for imaging deeper in tissues. The Science and Technology Center at Carnegie Mellon University will then develop methods to derivatize Qdots for existing and new applications in cell biology. The STC will initially use available Qdots and conjugates, label cells by a variety of means, then test the cells for fluorescent brightness, stability of labeling, and cell survival and function. As newer quantum dots become available, they will be similarly tested and compared with existing Qdots and existing organic fluorescent probes, e.g., Alexa dyes and cyanine dyes. Fluorescence lifetime imaging capability will be added to the 3-D grating imager in the STC for enhancing signal-to-background in deep tissue imaging by taking advantage of the relatively long emission lifetime of Qdots.
To obtain feedback for the development program we have included collaboration with the recently formed Bone Tissue Engineering Center at Carnegie Mellon University. In this collaboration we will examine the utility of the developing Qdot 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 Qdot technology to extend cell tracking to denser and more highly scattering tissue matrices, including hydroxyapatite-containing artificial bone matrices. By the conclusion of this project, we expect to have instrumentation and probes to perform time-resolved multicolor imaging at millimeter depth in many natural and artificial tissues. Thus the technology will be generic and have utility 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. Quantum Dot Corporation has been an effective partner in both producing quantum dots and in devising new surface coatings for use in biology. During the past two years we have used several different surface coatings to stabilize quantum dots for use in aqueous solution. By modifying these surfaces, we have:

(1) Greatly increased spontaneous cellular uptake of Qdots in vitro (Lagerholm *et al.,* submitted for publication; this method has been commercialized by Quantum Dot Corporation.)

(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 with much greater ease than hitherto possible. Details of vascularization and neovascularization were made visible with unprecedented sensitivity and clarity. Nascent and very small capillaries were clearly displayed. Cyanine dyes were easily usable with Qdots.

a. Use of multicolor fluorescence enabled simultaneous investigation of vasculature and matrix invasion. Quantum dot-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.

b. We investigated several different methods of fixation, in order 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.

c. 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-angiogensis 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.)

(4) Coupled quantum dots to biological materials for labeling and targeting. Our coated quantum dots retain their fluorescence *in vivo* for at least one year.

(5) Extended emission wavelengths of our quantum dots to the near infrared (850nm emission), with good stability and quantum yield. These infrared quantum dots allow deeper imaging *in vivo* and in tissue matrices.

(6) Used several new methods for insulating the core/shell Qdot nucleus from aqueous environments, and incorporated Foerster energy transfer reagents into the coatings. These systems could potentially be useful as *in vivo* sensors.

ISSUES

We have no significant issues in our partnership. Personal visits, emails, and weekly phone conferences have ensured that communication and exchange of materials have been easy and effective.

PI: William R. Wagner, PhD.
McGowan Institute for Regenerative Medicine
University of Pittsburgh
100 Technology Dr., #226
Pittsburgh, PA 15219
T: (412) 235-5138
F: (412) 235-5110
wagnerwr@upmc.edu

PROJECT TITLE: Cardiopulmonary Organ Engineering

PARTNERS' NAMES AND AFFILIATIONS:
Johnny Huard (Children's Hospital of Pittsburgh); Robert Kormos (Surgery, Univ. of Pittsburgh); Michael Sacks (Bioengineering, Univ. of Pittsburgh); Mark Pittenger (Osiris Therapeutics); David Vorp (Surgery, Univ. of Pittsburgh); Flordeliza Villanueva (Medicine, Univ. of Pittsburgh); Simon Watkins (Cell Biology and Physiology, Univ. of Pittsburgh)

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 much of our work in the first year has focused on developing our rat surgical model and then proceeding to investigate our best scaffold to date (developed by the Matrix Synthesis Core) as a full thickness replacement of the rat right ventricular outflow tract. Briefly, we utilized a biodegradable poly(ester urethane)urea 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. 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. Early results from this work show some evidence of MDSC differentiation to or fusion with cardiomyocytes, but further experimentation is needed.

For the construction of a tissue engineered vascular graft (TEVG), we have performed experiments where small, three dimensional tissue strips that can be stretched in our existing Flexcell apparatus. The preliminary results from these experiments showed that the constructs withstood the straining regimen without breaking and compaction was achieved. In order to create a TEVG capable of perfusion in our TEVG bioreactor, we have employed several methods. We have attempted to utilize a pure collagen gel seeded with cells, a naturally derived extracellular matrix derived from the urinary bladder mucosa, and have also investigated the use of fibrin gel (as well as combinations of these approaches). Our first construct containing the vacuum dried urinary bladder mucosa covered by a fibrin gel containing 10^6 cells achieved a burst pressure of 114mmHg, which was improved over previous techniques, but not adequate. In this work the UBM did not rupture, and the failure to achieve higher burst strength was due to an inadequate seal on the UBM, allowing fluid to leak around the vessel and break through the fibrin gel. We have since begun alterations to the vacuum drying technique to remove flaws in the sealing process as well as utilizing non-resorbable sutures and a fibrin glue used clinically to seal vascular leaks. In work focused on differentiating precursor cells to vascular cells, we have performed cyclic stretch and pressure experiments using rat and porcine bone marrow derived progenitor cells, as well as MDSCs. The results of these experiments have generally shown that each of the cell types is responsive to both cyclic pressure and cyclic stretching by changes in proliferation, alignment, and/or morphology, although definitive differentiation to smooth muscle cells has not yet been demonstrated.

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

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

ISSUES

Our first year of activity has been productive and we are establishing effective collaborative mechanisms and an effective administrative reporting structure.

PI: Herbert Waite, Ph.D.
University of California, Santa Barbara (UCSB)
MSI
Tr. 338, Lagoon Road
Santa Barbara, CA 93106
T: 805-893-2817
F: 805-893-7998
waite@mail.lifesci.ucsb.edu
http://www.lifesci.ucsb.edu/mcdb/faculty/waite/research/research.html

PARTNERS' NAMES AND AFFILIATIONS:
Galen Stucky (Chemistry, UCSB), Frank Zok (Materials, UCSB),
Tom Soh (Mechanical Engineering, UCSB)

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

PROJECT TITLE: Biomimetic Blades: Mincing with less Mineral

ABSTRACT
Tooth enamel and dentin are the premier materials in vertebrates for hardness and abrasion resistance. The superb properties of these materials are vital adaptations for proper ingestion nutrition and, when compromised through decay or injury, pose many fundamental and technical challenges to effective restoration. In polychaete worms such as Glycera and Nereis, the tooth-like jaws have a resistance to wear that is comparable to enamel; however, this is accomplished with a tenth as much mineralization (Glycera) or no mineralization at all (Nereis). We believe that these mainly proteinaceous jaws offer important insights into the design of biocompatible wear-resistant materials. Based on preliminary studies, we propose to demonstrate that specific proteins/polymers can be hardened and toughened by mineralization, metal ion chelation, or both. Our aim in this discovery-driven proposal is a state-of-the-art chemical, structural and mechanical characterization of the jaws using mass spectrometry, molecular biology, X-ray analysis and nanoindentation. Rigorous engineering principles will be applied to the analysis of jaws to distill a set of biomimetic rules regarding the relationship between structure and wear. Significant correlations between the chemical, microstructural and mechanical properties will be used to direct the preparation of His-containing copolymers into hard films containing Cu or Zn ions. The chief health benefits of this research will be insights about lightweight replacement materials with superior hardness and abrasion resistance.
Aim 1: Chemical, structural and mechanical investigations of Glycera and Nereis jaws shall elucidate the composition, protein sequence and the 3-dimensional organization of the components and shed light on their relation to the exceptional mechanical properties of this biomaterial.
Aim 2: In a second step, the Zn-based Nereis jaws shall be exploited as a paradigm to produce Zn-hardened biomimetic films. Since Zn is much more biocompatible than Cu, preference will be given to imitating the Zn-based Nereis jaw material rather than the Cu-based Glycera jaws, especially in view of possible medical biomaterials.

STATUS OF RESEARCH AND PARTNERSHIP

We have made significant progress on the first aim during the past year. In Nereis jaws, elemental and mechanical maps have been completed. These include maps of the halogens iodine, bromine and fluorine, which are limited to the outer periphery and the base. Over 15 halogenated amino acids have been identified from acid hydrolyzed material: the novel ones include mono- and dibromotyrosine, diiodotyrosine, dichlorotyrosine, dibromodi- and trityrosine, and dibromo- and diiodohisitidine. These are presumably components of a protein localized to the cuticular outer layer on the jaws. The mechanical maps are characterized by gradients having the highest stiffness and hardness around the tip and serrations. These are the same regions with elevated Zn content. Proteins in the core of the Nereis jaw are histidine rich. One prominent protein having a mass of about 40 kDa has been isolated, and peptides derived by trypsin digestion are being sequence by tandem MS.

In Glycera jaw, we were surprised to find some fundamental biochemical differences. Although these also have an associated histidine-rich protein (mass 37 kDa), the jaw stability towards acids and bases is notably higher than that of Nereis. A significant residue (~30% weight) resists even hydrolysis in concentrated sulfuric acid for 48 hrs. This residue appears to be structural melanin and intimately associated with the histidine-rich protein. Nanoindentation maps of stiffness and hardness show that these are closely linked to the distribution of copper.

ISSUES

Research: We see the necessity of reconfiguring the indentation methodology so that measurements can be done with completely hydrated specimens. This presents some interesting challenges for largely organic specimens.

Administration: Overcoming initial inertia and getting up to speed with the full parcel of personnel has been a challenge in our first year due to searches and recruitment delays. We now have four full-time grad students and four post-docs in addition to the four co-PIs. An administrative assistant is about to be designated.

Program: Getting good intercommunication with partners is the major issue with our project. We have begun to address this with weekly informal discussion meetings and lab rotations for all students and post-docs.

PI: Jonathan Wall, PhD
Human Immunology & Cancer Program
University of Tennessee Graduate School of Medicine
1924 Alcoa Highway
Knoxville, TN 37920-6999
T: (865) 544 9165
F: (865) 544 6865
jwall@mc.utmck.edu

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

PARTNERS' NAMES AND AFFILIATIONS:
Dr. Mike Paulus, Dr. Shaun Gleason & Dr. Steve Kennel
Oak Ridge National Laboratory
PO Box 2008 MS 6006
Oak Ridge, TN 37831-6006

Dr. Jens Gregor
The University of Tennessee
Department of Computer Science
1122 Volunteer Blvd , Suite 203
Knoxville TN 37996-3450

Dr. Robert Donnell
The University of Tennessee
Pathology
A201 Veterinary Teaching Hospital
2407 River Drive , Knoxville

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

At the end of yr. 1 we were beginning our initial studies using I-125 labeled serum amyloid P-component (SAP) as a tracer in mice with severe systemic AA-amyloidosis – our goal was to validate its use as an amyloid tracer in mice and compare its activity to that of 11-1F4 (presumed a priori to be an effective AA-amyloid tracer – see **Issues** below). Additionally, a suite of correlative protocols was being developed that would support the image datasets and confirm the co-localization of radiotracer with amyloid in target organs- these included biodistribution and micro-autoradiographic analyses. We have performed a number of studies with ^{125}I-SAP and demonstrated conclusively its co-localization with AA amyloid deposits in the liver, spleen, heart, pancreas and kidneys. In addition we have generated high-resolution co-registered SPECT/CT images that unequivocally identify amyloid deposits in the liver and spleen. The correlative studies have confirmed our interpretation of the image data. The primary objective for the hardware development component of this program during Year 2 was to characterize the microSPECT/CT system developed during Year 1. The major Year 2 imaging milestones are summarized as follows: i) the microSPECT imaging system spatial resolution and sensitivity were characterized using low energy, high-resolution parallel hole and pinhole collimators; ii) SPECT and CT phantom studies were performed and reconstructed images were registered; iii) More than 50 mice have been imaged using a variety of amyloid-targeting I-125 labeled compounds. Additionally, the design of improved detectors and associated electronics is underway and will be completed during Year 2 using Hamamatsu 8500 multi-anode photomultiplier tubes coupled to pixilated CsI. The new detector system will be added to the existing microCT platform at the end of Year 2 or the beginning of Year 3. The continuous development of software has enhanced our ability to rapidly reconstruct and quantitatively analyze the large SPECT and CT datasets. These improvements include: work on the modular framework for iterative reconstruction; a parallelized version of the EM-ML algorithm that supports both parallel-hole and pinhole collimation, and; an automated, model-driven method for accurate segmentation of organs in images

ISSUES

Once again we had to convince the lawmakers of Tennessee that the sub-contract with Oak Ridge National Laboratory could be “sole-sourced” rather than put out to bid. This led to a 6 month delay in getting funds to the Laboratory. Scientifically, we have found that our initial plans to use the 11-1F4 antibody to label amyloid deposits in vivo are no longer viable; therefore we have been evaluating other candidate reagents. Otherwise, we have not encountered any major problems.

PI: Lihong Wang, Ph.D.
Optical Imaging Laboratory
Texas A&M University
3120 TAMU (233 Zachry Bldg.)
College Station, Texas 77843-3120, USA
T: 979-847-9040
F: 979-845-4450
LWang@tamu.edu
http://oilab.tamu.edu

PARTNERS' NAMES AND AFFILIATIONS:
George Stoica (Texas A&M University) and Quing Zhu (University of Connecticut)

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

PROJECT TITLE: Functional brain imaging by laser-induced PAT

ABSTRACT

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 diffraction-limited high acoustic resolution, the proposed technology, functional photoacoustic tomography (fPAT), can image the intact brain free of speckle artifacts. Besides structural information, the proposed fPAT can also provide functional information including blood volume and blood oxygenation.

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

Optical contrast is sensitive to the molecular conformation of biological tissue and is related to certain physiological parameters such as the level of hemoglobin oxygenation. The proposed fPAT technology combines the high-contrast advantage of optical imaging with the high 3D resolution advantage of ultrasound imaging. The proposed technology does not depend on ballistic/quasi-ballistic or backscattered light as optical coherence tomography (OCT) does. Any light, including both singly and multiply scattered photons, contributes to the imaging signal; as a result, the imaging depth in fPAT is better than in OCT. The resolution is diffraction-limited by the detected photoacoustic waves rather than by optical diffusion; consequently, the resolution of fPAT is excellent (60 microns, adjustable with ultrasonic frequency). Furthermore, fPAT is free of the speckle artifacts present in OCT and pulse-echo ultrasonography, two analogous technologies.

STATUS OF RESEARCH AND PARTNERSHIP

In the first year of this project, we performed functional photoacoustic imaging of rat brains *in vivo* to demonstrate the capability of PAT to assess simultaneously cerebral blood volume and oxygenation. Transcranial imaging of two functional parameters, the total concentration of hemoglobin and the oxygen saturation of hemoglobin, in small-animal brains was realized *in vivo* non-invasively by laser-based photoacoustic tomography for the first time to our knowledge. Multi-wavelength spectroscopic photoacoustic tomography can assess the optical absorptions of endogenous chromophores, e.g., oxygenated and deoxygenated hemoglobin, while its spatial resolution is diffraction-limited by the photoacoustic signals rather than by optical diffusion. This technique, with its prominent intrinsic advantages, can potentially accelerate the progress in neuroscience and lead to a better understanding of the interrelationship between neural, hemodynamic and metabolic activities in the brain.

ISSUES

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

PI: Richard F. ff. Weir, Ph.D.
Research Assistant Professor
Physical Medicine & Rehabilitation
Northwestern University Prosthetic Research Laboratory
345 E. Superior, RM1441
Chicago, Illinois 60611
T: (312) 238-6512
F: (312) 238-6510
rweir@northwestern.edu
http://pubweb.northwestern.edu/~rfw586

PROJECT TITLE: Multifunction Prosthesis Control using Implanted Sensors

PARTNERS' NAMES AND AFFILIATIONS:
Philip R. Troyk, PhD, Illinois Institute of Technology, Chicago, Illinois
Glenn A. DeMichele, MS, Douglas A. Kerns, PhD, Sigenics Corp., Lincolnshire, Illinois
Todd A. Kuiken, MD, PhD, Rehabilitation Institute of Chicago, Chicago, Illinois

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

ABSTRACT
The limitation of current prostheses is not the devices themselves but rather the lack of sufficient independent control sources. A system capable of reading intra muscular EMG signals would greatly increase the number control sources available for prosthesis control. Current state-of-the-art electric prosthetic hands are generally single DOF (opening/closing) devices often implemented with EMG control. Current prosthetic arms requiring multi-DOF control most often use sequential control. As currently implemented, sequential control is slow.
We propose to develop a multichannel/multifunction prosthetic hand/arm controller system capable of receiving and processing signals from up to sixteen implanted bipolar differential electromyographic (EMG) electrodes. An external prosthesis controller will use fuzzy-logic to decipher user intent from telemetry sent over a transcutaneous magnetic link by the implanted electrodes. The same link will provide power for the implanted electrodes.
- Northwestern University will develop the multifunctional prosthesis controller and perform the animal experiments necessary to demonstrate the implanted devices.
- Rehabilitation Institute of Chicago will perform animal experiments and help with human subject experiments.
- Illinois Institute of Technology will develop individually addressable integrated circuit EMG sensor packages. Each sensor will be housed in BION® hermetically sealed packages provided by the Alfred E. Mann Foundation.
- Sigenics Corp. will develop the transcutaneous telemetry link, (or reader). A custom-designed application-specific integrated circuit (ASIC) will "strip" the data from the link's telemetry and send it to the prosthesis controller. Powering of the implanted electrodes will also be controlled by the ASIC. The external coil of the inductive link will be laminated into a prosthetic socket.
Development of each component of the system will occur in parallel. Throughout years 1 & 2 fine wire studies with human subjects will be used to develop multifunctional prosthesis control algorithms. Initial silicon for the implanted electrodes and reader ASIC will be ready by end of year 1. Packaged electrodes ready for animal testing and a prototype reader will be ready the middle of year 2. Year 3 is expected to be spent going through initial system integration and iterative test-redesign cycles. A definitive design is anticipated to be ready for final testing and tweaking by the middle of year 4. The final year will be spent conducting the final systems integration.

STATUS OF RESEARCH AND PARTNERSHIP

Northwestern with help from the Rehabilitation Institute of Chicago is responsible for performing fine wire intra-muscular experiments on human subjects during the first year of this project to acquire data to develop a prosthesis controller capable of handling up to 16 inputs. This data, is acquired from up to 10 muscles simultaneously in 3 normal and 3 amputee subjects, is to be used for algorithm development for the prosthesis controller. This controller will ultimately receive data of a similar nature over a telemetry link, being developed by Sigenics, from the implanted electrodes being developed by IIT. Based on this data the controller will decide which motors in the prosthesis to actuate.

So far we have acquired data from 2 subjects and we still in the process of post-processing this data. The remaining subjects are scheduled and will take place over the course of the summer. The human subject protocol was revised to allow us to collect kinematic data of the hand and wrist (through the use of our motion capture laboratory), along with the previously approved collection of EMG data, while subjects perform simple reach-to-grasp movements. This was done to allow us to explore the relationship between extrinsic hand muscle activity and the kinematics of the hand during reaching and grasping.

The hardware development by IIT and Sigenics proceeds apace and is also making good progress. An initial silicon application-specific-integrated circuit (ASIC) design has been submitted for a June 14, 2004 XFab CX08 engineering wafer run. A number of technical issues relating to reverse telemetering data over a Class-E Power Converter have been resolved and exploration of small size and light weight reader coil materials are being explored. The size, weight and power consumption of the reader coil was one of the key technical issues to be resolved in this project. During this first year of the BRP, tasks performed by IIT and Sigenics were coordinated so as to optimize the use of expertise at the two partners. Design of the combined implant/external controller system must be accomplished using a top-down system-level approach. A system architecture has been developed and a silicon application-specific-integrated circuit (ASICs) is to be fabricated to demonstrate the feasibility of the system approach. Progress for each of the system modules targeted for feasibility study is reported below.

In other work, leveraging off of Dr Kuiken's NICHD Field Initiated Research Grant #R01 HD043137-01 "Analysis of EMG propagation in planar muscles for improved prosthesis control" (12/02-11/07), the pick-up volume of a BION-like recording electrode was estimated using a model of the intramuscular EMG signal.

ISSUES

BRG/BRP's are a very valuable funding mechanism. A major advantage of the BRP format is that it can be development oriented and it enables this kind of technical development by allowing people from many disparate backgrounds to come together to solve a particular problem. No other grant mechanism offers quite this same potential.

PI: Shimon Weiss
Department of Chemistry and Biochemistry; Department of Physiology
University of California at Los Angeles
P.O. Box 951569
607 Charles E. Young Drive East
Lost Angeles, CA 90095-1569
T: 310-794-0093
F: 310-267-4672
sweiss@chem.ucla.edu

PROJECT TITLE: Development of Q-dots as biological probes

PARTNERS' NAMES AND AFFILIATIONS:
Prof. Paul A. Alivisatos, Co-PI
Department of Chemistry
University of California at Berkeley
Berkeley, CA 94720
E-mail: alivis@uclink4.berkeley.edu, Tel: 510-643-7371, Fax: 510-642-6911
Prof. Hsiao-Ping Moore
Department of Molecular and Cell Biology, University of California at Berkeley
Berkeley, CA 94720-3200
E-mail: hpmoore@uclink4.berkeley.edu, Tel: 510-643-6528, Fax: 510-643-8708
Prof. Carolyn Larabell
Department of Anatomy
University of California at San Francisco
E-mail: larabel@itsa.ucsf.edu, Tel: 415-514-0423, Fax: 415-476-4845
Prof. Sam Gambhir
Department of Molecular & Medical Pharmacology; The Crump Inst.
University of California at Los Angeles
E-mail: sgambhir@mednet.ucla.edu, Tel: 310-206-1798, Fax: 310-209-4655

GRANTING NIH INSTITUTE/CENTER: NIBIB (lead), NIGMS, NCI

ABSTRACT
The long-term goal of this Bioengineering Research Partnerships is to develop semiconductor nanocrystals fluorescent probes (Q-dots) technology that will provide biomedical research with better tools for diagnosis of diseases and biomedical techniques and instrumentation necessary for basic research of cellular and molecular structure and fundamental life processes. This includes Q-dot probe synthesis, bio-conjugation techniques, dedicated optical instrumentation and unique imaging methodologies. We will develop optimized protocols for Q-dot synthesis with desired optical, physical and chemical properties. Various spectroscopic and structural measurements will be used to fully characterize Q-dots. This information will be fed back into the synthesis for optimization of the desired properties. Bio-conjugation schemes and labeling protocols will be developed for biomolecules in fixed and living cells.

STATUS OF RESEARCH AND PARTNERSHIP
1. Development of new qdots for NIR imaging and single-molecule particle tracking in live cells: We have fully characterized the peptide coating approach. We are currently working on alternative peptide sequences to enhance diverse qdots properties. We demonstrated increased photostability and high brightness of peptide coated CdSe cores / CdS/ZnS graded shells and used these particles for single molecule live cell trafficking measurements. We have observed red shift of the emission peak, attributed

to interaction of the excitonic wavefunction with the peptide molecular orbitals. We also synthesized high brightness Type-II IR qdots and successfully coated them with peptides. These IR emitting particles are suitable for background-free imaging of live cells in tissue cultures and in small animals.
Development of new types of nanoparticles and nanoparticle complexes: We developed colloidal tetrapods and hollow metal oxide qdots with possible drug delivery uses and Au-semiconductor conjugates with unique photophysical properties governed by exciton-plasmon interactions.
2. Dual-color TIRF single-molecule setup: In addition to our commercial confocal and wide-field deconvolution microscopes, we have modified one of our single-molecule setup for dual-color total internal reflection fluorescence microscopy (TIRF). This setup uses an electron-multiplied CCD with single-photon sensitivity, and specifically developed software allowing us to track individual qdots in the basal membrane of live cell and correlate trajectories with domain-specific membrane staining.
Whole animal imaging of NIR qdots: We modified an existing IVIS Animal Imaging System to have an efficient excitation and detection of IR qdots in mice.
3. FISH studies using DNA oligonucleotide functionalized qdots: Using commercially available streptavidin-coated qdots, we have designed a strategy to create FISH probes using biotynilated DNA oligonucleotides. Proof of principle of single excitation/multicolor detection of various repeated sequences was performed on metaphase and interphase chromosomes.
Development & characterization of avidin fusion constructs for receptor trafficking studies: We have made several avidin constructs by fusing chicken avidin to the targeting sequences of specific organelles. A particular useful construct is a GPI-anchored avidin fused to the targeting sequences of human CD14. This construct is targeted to cholesterol-sensitive lipid rafts as shown by floatation assay of detergent-resistant membranes and was used to stably transfect Hela cells. For comparative studies, we have made another construct (furin-avidin) that cycles between the TGN and the cell surface like GPI-anchored avidin but is not expected to localize to lipid rafts. We are characterizing the oligomeric states of the avidin constructs, and are investigating the signaling cascade that influences the distribution/dynamics of lipid microdomains.
Single-molecule tracking of qdot-labeled chimeric receptors in live cell: We used biotynilated peptide-coated qdots to target GPI-anchored CD14-avidin chimeric receptors expressed in the plasma membrane of HeLa cells to study lipid rafts and their associated proteins. The high photostability of the qdots allowed tracking single receptors over minutes with <100 ms and <50 nm resolution. Dual-color TIRF imaging revealed the dynamic interactions of the receptors with cholera toxin-Alexa 488 labeled sphingolipid/sterol-rich microdomains. Distinct diffusion patterns and diffusion coefficients of the receptors were observed and correlated with the distribution of the microdomains in the cell membrane.
Study of cancer cell invasion of prototype organoids: We used previously reported qdot invasion assay to compare the behavior of 7 different adherent human cell lines, including breast epithelial MCF 10A, breast tumor MDA-MB-231, MDA-MB-435S, MCF 7, colon tumor SW480, lung tumor NCI H1299, and bone tumor Saos-2, and observed two distinct behaviors of cancer cells that can be used to further categorize them. Some cancer cell lines demonstrated fibroblastic behaviors and left long fluorescent-free trails as they migrated across the dish, whereas other cancer cells left clear zones of varying sizes around their periphery. We extended these studies to 3D cultures in which cells can recapitulate growth of super-cellular structures in a way that resembles natural glandular formation.
Live mice imaging of NIR qdots: Three models are considered to achieve non-invasive imaging of tumor targeting in living animals utilizing qdots: (i) sensitized T lymphocyte mediated tumor targeting, (ii) anti-tumor antibodies for tumor targeting and (iii) biotinylated qdots for targeting avidin expressing cells. We have established tumors in nude mice using Hela cells that are stably transfected with CD14-Avidin. However targeting in these approaches suffered due to hepatic trapping of qdots. We are presently investigating the use of PEGylated qdots as a means to improve the half-life of qdots in blood.

ISSUES
We are into the 5th of this grant. We wonder what should be the mechanism for BRP renewal.

PI: John S. Werner, Ph.D.
Department of Ophthalmology
Section of Neurobiology, Physiology and Behavior
University of California Davis
4860 Y St., Suite 2400
Sacramento, CA 95817
T: 916-734-6817
F: 916-734-4543
jswerner@ucdavis.edu
http://trc.ucdavis.edu/psychophysics/

PARTNERS' NAMES AND AFFILIATIONS:
Scot Olivier (Lawrence Livermore National Laboratory), Don Miller (Indiana University)

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

PROJECT TITLE: Ophthalmic Imaging Using Adaptive Optics and OCT

ABSTRACT
The purpose of this BRP is to develop and evaluate new optical instrumentation that will permit unprecedented three-dimensional, in vivo, imaging of single cells in the human retina, specifically rod and cone photoreceptors and ganglion cells. An interdisciplinary team will combine 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 has been funded for 8 months and has already made excellent progress. A core system, combining AO with en face scanning laser imaging, has been designed and will soon be constructed and tested. The main features include a selectable source (630 nm laser diode or 842 nm super luminescent diode) to create a point image on the retina, a Hartman-Shack wavefront sensor (100 lenslets) to detect the aberrations of the eye, and a deformable mirror (DM) to correct the aberrations in real time. X-Y scanners are used to create near diffraction limited images over a 3° region of retina. Image acquisition is at video rates with 512 x 512 pixels per frame.

The scan beams are relayed between elements using spherical mirrors to avoid back reflections that would otherwise mask the weak retinal reflections. The telescopic-relay magnifications are based on the physical sizes of the X-Y scanners and the deformable mirrors. Scanners, deformable mirrors and wavefront sensor are placed in planes conjugate with the pupil of the eye. A low-noise science CCD camera is placed in a plane conjugate with the retina. An optical trombone is introduced to permit manual focus up to 300 μm within the retinal layers.

A secondary arm provides a lower resolution, 30°-retinal viewfinder to aide in navigating about the retina and locating areas of interest to be imaged at high resolution. This arm includes a fundus camera with a halogen source, a fixation target and a pupil camera for eye alignment.

AO and OCT are each complex technologies so we have decided to build each system separately before attempting to bring them together. OCT continues to develop rapidly with major technological advances since this BRP was submitted for review in 2002. An evaluation of new approaches has led us to pursue Fourier-domain OCT rather than time-domain OCT as originally proposed. The main advantages of the Fourier-domain, or spectral, OCT are substantially higher A-scan acquisition rates and greater signal-to-

noise than is possible with time-domain OCT. Our first-generation OCT system uses a super luminescent diode (SLD) whose near-infrared wavelength (842 nm) is comfortable to the patient. The SLD light is directed into reference and sample arms. The two reflected beams are combined and imaged onto a (1200 1/mm) diffraction grating and then onto a 12-bit line-scan CCD camera. Cellular structure is reconstructed in depth from the interference pattern recorded by the CCD using an inverse Fourier transform and signal-processing algorithms carried out on a lab computer. With this system, in vivo structures have been imaged in real time with a sensitivity of 82-102 dB (10-8.2 to 10-10.2 of the incident power entering the eye) per 100 μs exposure for one A-line. The current system operates at 10,000 A-lines/s, however, only 3-4 frames/s (each consisting of 1000 A-lines) are streamed to disc and the other frames are dropped. We expect that more efficient algorithms being developed will allow us to use all frames. The current system has ~6 μm axial resolution and ~16-20 μm lateral resolution in retina. The lateral resolution will be increased by use of AO and the axial resolution will be increased by obtaining a broader bandwidth source.

ISSUES

A central limitation in AO applications for vision science is that currently available deformable mirrors (DMs) have limited dynamic range (stroke). Our evaluation of liquid crystal spatial light modulators demonstrated performance limitations due to their slow update rate and calibration instability. Our current design cascades two DMs; one is a high-stroke bimorph with 37 actuators in a radial pattern that is well suited for correction of lower-order aberrations, and the second is a 140-actuator DM for higher-order aberration correction. Before incorporating each DM, it will be evaluated with an interferometer and performance will be compared in an AO flood-illumination system with a 109-actuator conventional DM.

PI: Dwayne Westenskow PhD
Professor and Director of Bioengineering
Department of Anesthesiology
30 North 1900 East Room 3C444
University of Utah
Salt Lake City, UT 84132
T: 801-581-2478
Dwayne.Westenskow@hsc.utah.edu

PARTNERS' NAMES AND AFFILIATIONS:

Westenskow, Dwayne R, PhD	Univ of Utah, Dept of Anesthesiology
Agutter, James, M.Arch.	Univ of Utah, Dept of Architecture
Bermudez, Julio, Ph.D.	Univ of Utah, Dept of Architecture
Drews, Frank, Ph.D.	Univ of Utah, Dept. of Psychology
Egan, Talmage D., M.D.	Univ of Utah, Dept of Anesthesiology
Johnson, Kenward, M.D.	Univ of Utah, Dept of Anesthesiology
Strayer, David, Ph.D.	Univ of Utah, Dept of Psychology
Syroid, Noah, M.S.	Univ of Utah, Dept of Anesthesiology

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

PROJECT TITLE: Data Display to Detect–Diagnose–Treat Critical Events

ABSTRACT
A Bioengineering Research Partnership at the University of Utah has developed new methods of presenting patient information to the clinician with the goal of improving patient safety. This interdisciplinary partnership of biomedical and computer engineers, information architects, clinicans, and human factors experts has developed three core technologies:

- modeling of patient's physiology and pharmacology,
- information visualization of model-based and sensor-based patient information,
- and human factors centered evaluation of new medical information displays.

The displays incorporate information based on physiologic and pharmacologic models, providing patient care data that are currently unavailable in everyday practice. With this approach, the new display technologies support the caregivers' mental representation of the care system and address their real-time clinical needs thereby improving quality and safety of care.

STATUS OF RESEARCH AND PARTNERSHIP:
During the past five years, our Bioengineering Research Partnership has developed core technologies for modeling and visualizing pharmacologic drug effects and cardiovascular and pulmonary physiology. The topics covered by this work include:

- Methods for user-centered, object-oriented display design and testing.
- Low and high-fidelity simulation environments for:
  - Measurement of critical event detection time,
  - Measurement of treatment efficacy,
  - Measurement of display utility using eye-tracking and work load assessment.
- Models of cardiovascular and pulmonary physiology,
- Drug pharmacokinetic and pharmocodynamic models to predict anesthesia effects,
- Methods to validate models in animal, volunteer, and clinical studies.

Pharmacologic modeling has been successfully incorporated into a display that supports drug management in the operating room. Preliminary clinical studies indicate that the models adequately predict the clinical effects of intravenous anesthetics and improve clinical care. Graphical presentation of clinical information improves a clinician's ability to detect, diagnose, and treat cardiovascular events. The simple shape and texture changes associated with the ST object in a cardiovascular display provided an emergent feature that gave the clinician a salient indication that something specific was wrong. The graphical display resulted in more effective treatment, evidenced by less deviation of arterial blood pressure from baseline, CVP closer to its baseline, and SpO2 higher at the end of the case. Two emergent features shown in a pulmonary display helped anesthesiologists rapidly differentiate obstructed endotracheal tube from bronchospasm. The pulmonary model transformed monitored variables into measurements of airway resistance and the display brought their abnormal values to the attention of the study subjects. We plan to combine the cardiovascular and the pulmonary displays to meet the needs in several clinical environments, including support for clinician-clinician communication.

Our Bioengineering Research Partnership has developed graphic displays to support anesthesia drug management and the detection of critical events in the operating room. Anesthesiologists using the drug display provided better pain management and patients emerged faster, without hemodynamic complications. When the cardiovascular and pulmonary displays were used by anesthesiologists, simulated life-threatening events were detected and treated significantly faster. Anesthesiologists detected the evolution of myocardial ischemia almost three times faster than when not using the displays. This led to an equally impressive improvement in the time taken to initiate treatment (2.6 vs. 5.1 minutes, see Appendix A6). Anesthesiologists using the respiratory display correctly treated an obstructed endotracheal tube 40% faster. These two findings have clinical significance. As outlined in advanced cardiac life support guidelines, ensuring a patent airway and the prompt detection of ischemia reduces the extent of cardiac injury and mortality following a life-threatening event. The current displays were designed to support anesthesiologists, and substantial modifications may be needed to support other care providers. In addition, many of these results were obtained in a simulated environment and must be confirmed under actual clinical conditions.

ISSUES
None.

PI: Stephen H. White
University of California at Irvine
Dept. of Physiology and Biophysics
Med. Sciences I, D346
Irvine, CA 92697-4560
T: 949-824-7122
blanco@helium.biomol.uci.edu

PROJECT TITLE: Cold Neutrons for Biology and Technology

PARTNERS' NAMES AND AFFILIATIONS: University of California at Irvine, National Institute of Standards and Technology, Rice University, Carnegie Mellon University, Duke University, University of Pennsylvania, and Johns Hopkins University.

GRANTING NIH INSTITUTE/CENTER: National Institute for Research Resources

ABSTRACT
The Cold Neutrons 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% 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 the 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 reflection/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

The project is healthy and on course. The following tasks have been accomplished: (1) Director appointed, (2) AND/R construction has been completed, (3) the tests and trials of AND/R are nearly complete, (4) Instrument scientist appointed, (5) Programmer appointed, (6) two postdoctoral students appointed, (7) distinguished visiting scientist appointed for 1 year to establish new diffraction methods, (8) web-based purchasing system established, and (9) PC clusters for molecular dynamics simulations have been installed and brought to operational status at UC Irvine and NIST Center for Neutron Research. Each co-PI has is actively engaged in research using the AND/R. In addition, Dr. Loesche (CNBT Director) has an active outreach program that is recruiting new users to neutron researcn.

ISSUES

None.

PI: David R. Williams, Ph.D.
University of Rochester
Center for Visual Science
274 Meliora Hall
Rochester NY 14627-0270
T: 585-275-8672
F: 585-271-3043
david@cvs.rochester.edu
http://www.bcs.rochester.edu/people/williams/

PROJECT TITLE: Adaptive Optics Instrumentation for Advanced Ophthalmic Imaging

PARTNERS' NAMES AND AFFILIATIONS:
David Williams, University of Rochester, Rochester NY
Scot Olivier, Lawrence Livermore National Laboratory, Livermore CA
Srinivas Sadda, Doheny Eye Institute, University of Southern California, Los Angeles CA,
Austin Roorda, University of Houston, Houston TX
John Flannery, University of California, Berkeley CA
Steve Burns, Schepens Eye Research Institute, Boston MA
R. Daniel Ferguson, Physical Sciences Inc.
Curtis Vogel, Montana State University

GRANTING NIH INSTITUTE/CENTER: National Eye Institute

ABSTRACT
The two goals of this Bioengineering Research Partnership are 1) to design and construct a new generation of instruments for noninvasive imaging of the mammalian retina with 3-D resolution superior to existing technology and capable of resolving single cells *in vivo*. 2) to explore the value of this technology through application to human retinal disease and retinal surgery. These instruments will combine adaptive optics, a technology borrowed from astronomy that automatically corrects all the eye's aberrations, with confocal microscopy, a technology for optically sectioning the retina. Devices will be deployed at each of four clinical sites: USC, Rochester, Houston, and Schepens. In years 2-5, these devices will provide high resolution imaging of neovascularization in age-related macular degeneration and diabetic retinopathy, photoreceptors in retinal degenerative disease such as retinitis pigmentosa, ganglion cell bodies in glaucoma, individual retinal pigment epithelial cells, and blood flow in the smallest retinal capillaries. In year 3, a new surgical microscope equipped with adaptive optics will be constructed by LLNL. Retinal surgeons at USC will evaluate this device in years 4-5. Based on its experience with earlier instruments, the BRP will design and build a sixth instrument in year 4 that will be portable, compact, and user friendly. This device will be available to investigators outside the BRP. The BRP brings together optical engineers, basic vision scientists, and clinical vision researchers. This will allow engineers to design instrumentation informed by the specific needs of clinical research, allowing them to translate adaptive optics technology directly into clinical application. LLNL brings to the partnership expertise in optical engineering and adaptive optics from the fields of astronomy and laser fusion. Rochester and Houston contribute experience in adaptive optics applied to retinal imaging. Rochester first applied adaptive optics to high resolution retinal imaging and Houston has recently demonstrated a prototype adaptive optics system that is the precursor for the devices proposed here. Schepens brings international leadership in scanning laser ophthalmoscopy. UC, Berkeley provides expertise in the study of retinal degenerative diseases. USC, with its innovative approaches to retinal disease and retinal surgery, will join Rochester, Houston, and Schepens in providing clinical sites for the evaluation of confocal adaptive optics technology. Physical Sciences and Montana State are providing technology for stabilizing image motion caused by movements of the eyes.

STATUS OF RESEARCH AND PARTNERSHIP

The design of the initial four instruments under development at Rochester, Houston, and SERI and LLNL has been completed and construction is underway. Rochester is already collecting retinal images with its instrument and Houston, SERI, and LLNL expect to have functioning instruments by the end of year 2. The rapid progress we have achieved so far can be partly attributed to extensive collaboration between investigators from the six participating institutions. All four instruments share a common core design that has been developed through six committees, each tasked with making design recommendations for a different subsystem in the instrument. Instrument development has received substantial input from the clinical researchers participating in the BRP to ensure that the devices will eventually be deployed in clinical research settings as well as the vision science laboratory.

ISSUES

A major concern in the development of all four instruments is that the availability of suitable deformable mirrors has been delayed. Deformable mirrors are the key components that allow high resolution retinal imaging without blurring from the eye's aberrations. The BRP plans to capitalize on the efforts of the Center for Adaptive Optics (CfAO), an NSF-funded Science and Technology Center, to develop a new kind of deformable mirror for vision science applications. CfAO has funded four different companies (Boston Micromachines, Lucent, Iris AO, and MEMX) to develop MEMS mirrors that will be smaller, cheaper, and have a larger dynamic range than current deformable mirror technology. Unfortunately, none of the companies have met their deadlines due to technical difficulties in fabricating these complex devices, creating a delay in the development of our instruments. However, Boston Micromachines has recently delivered a functional mirror. The dynamic range of this mirror is limited, which restricts how well it can correct large aberrations, but at least all four sites can develop an adaptive optics capability with this mirror. We anticipate that in future years we will need to invest in new mirrors, perhaps as often as yearly, as this technology improves.

PI: Karl Dane Wittrup
MIT 66-552
Cambridge, MA 02139
T: 617-253-4578
F: 617-258-5766
wittrup@mit.edu

PROJECT TITLE: Engineered Antibody EGFR Antagonist Cancer Therapeutics

PARTNERS:
Prof. Karl Dane Wittrup, Chemical Engineering & Biological Engineering, MIT
Prof. Douglas A. Lauffenburger, Chemical Engineering, BE, & Biology, MIT
Prof. Bruce Tidor, BE & Electrical Engineering & Computer Science, MIT
Prof. John Kuriyan, Chemistry & Biology, University of California, Berkeley

GRANTING NIH INSTITUTE/CENTER: National Cancer Institute

ABSTRACT
The objective of this BRP is to develop basic scientific understanding of aberrant signaling by epidermal growth factor receptor (EGFR) in cancer, and integrate this knowledge with development of new mechanism-based EGFR antagonists that will serve as therapeutic lead molecules. The partnership incorporates expertise and approaches from protein engineering (Wittrup), quantitative cell biology (Lauffenburger), computational biophysics (Tidor), and structural biology (Kuriyan). The importance of EGFR as a target in cancer has been validated recently by favorable clinical trial results for inhibitors against both its intracellular (Iressa, AstraZeneca) and extracellular (Erbitux, ImClone) domains.

STATUS OF RESEARCH AND PARTNERSHIP

1. Antibody Engineering. In the previous year, we have progressed on the following fronts: engineering the EGFR antigen to provide useful reagents for antibody isolation and affinity maturation; developing methodology for domain and residue-level epitope mapping; and isolation of antibodies directed against 6 specific peptides designed based on published structures of EGFR.

2. EGFR cell biology. The three major directions of effort during this past year and ongoing into the coming year are: [a] the effects of HER2 overexpression on cell signaling and functional responses, especially migration; [b] how autocrine EGFR ligands operate to regulate cell signaling and functional responses, especially migration; [c] measurement of protein phosphorylation in the EGFR system signaling network

3. Computational antibody design. Progress in computational modeling and design has progressed both in analyzing known antibody complexes and in developing and applying design methods for antibody-antigen interfaces. We have used theoretical simulations to compare the structure and energetics of the engineered antibody with the highest known affinity (4M5.3, with an affinity of 270fM) to its wild-type counterpart (4-4-20, with an affinity of 700 pM). The 1800-fold binding enhancement results from 14 mutations. The calculational analysis accounts for roughly one-third of the enhancement resulting from direct electrostatic interactions involving the mutated residues. The remainder appears to come from numerous small improvements. Together, the results illustrate a case where very high binding affinity is achieved through the cumulative effect of many small structural alterations.

4. EGFR structural biology. In order to gain a deeper understanding of the transmembrane signaling mechanism of EGFR, we are developing a mammalian cell system to express the intact EGFR for structural studies. We are currently optimizing the expression and purification procedures for preparing large-quantities of the proteins for protein crystallization trials.

Summary: Progress has been strong on all fronts, and further integration is expected in the coming year as high affinity antibodies become available from the antibody engineering component. These antibodies will be utilized in the receptor biology assays; binding epitopes will be predicted computationally; and these antibodies will be utilized as co-crystallization chaperones.

ISSUES

None.

PI: Jonathan R. Wolpaw, MD
Wadsworth Center
New York State Department of Health and State University of New York
Laboratory of Nervous System Disorders
P.O. Box 509
Albany, New York, 12201-0509
T: 518-473-3631
F: 518-486-4910
wolpaw@wadsworth.org
http://www.bciresearch.org

PARTNERS' NAMES AND AFFILIATIONS: Dennis J. McFarland, Theresa M. Vaughan, Gerwin Schalk (Lab Nerv Syst Disorders, Wadsworth Center); Andrea Kuebler, Niels Birbaumer (Inst Med Psychol & Behav Neurobiol, Eberhard-Karls-Univ Tuebingen, Germany); Melody M. Moore (Computer Informat Syst, Georgia St Univ, Atlanta, Georgia)

GRANTING NIH INSTITUTE/CENTER: NIBIB and NINDS

PROJECT TITLE: GENERAL PURPOSE BRAIN-COMPUTER INTERFACE (BCI) SYSTEM

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

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

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

STATUS OF RESEARCH AND PARTNERSHIP

The BRP's second year is proving to be extremely productive. Major progress includes:
(1) BCI2000, the general purpose BCI system, is now being used for: (a) sensorimotor rhythm-based BCI operation; (b) slow cortical potential-based BCI operation; (c) P300-based BCI operation; (d) BCI operation with activity recorded by electrode arrays on the cortical surface (electrocorticographic activity, or ECoG); (e) BCI operation using auditory (rather than visual) outputs; (f) BCI control of simple word-processing; and (g) BCI control of the multi-dimensional movements of a robotic arm. Thus, it is achieving the objective of a tool that greatly facilitates BCI research and development.
(2) The Albany and Tuebingen partners have successively trained all of an initial cohort of four patients severely disabled by ALS to control cursor movement using sensorimotor rhythms. These patients are now undertaking slow cortical potential-based BCI training. Additional patients are now entering the studying protocol. In tandem with this work, comprehensive and quantitative quality of assessment is also taking place.
(3) The Albany and Atlanta partners continue to progress in application of a sensorimotor-rhythm-based BCI to control of multi-dimensional movements. The control achieved by people using this non-invasive methodology is comparable in speed, precision, and accuracy to the control reported in monkey studies using electrodes implanted in cortex. People can use this control to reach novel as well as familiar targets. To date, the most successful users are two people confined to wheelchairs by spinal cord injuries. The two-dimensional control developed with the cursor is now proving capable of direct control of a robotic arm. A three-dimensional movement control protocol is under development.
(4) The Albany partner's collaboration with investigators at Washington University in applying BCI2000 for device control with ECoG signals from arrays placed on the cortex before epilepsy surgery has gone extremely well. The first results demonstrate remarkably rapid user mastery of cursor control, and also show that ECoG has high spectral and topographical resolution that should support rapid and accurate multi-dimensional control that may substantially exceed that achieved with scalp electrodes. Thus, minimally invasive cortical surface arrays may make it unnecessary to implant electrodes within cortex for multidimensional control.
(5) BCI2000 with documentation continues to be given to other research labs. About 25 labs are now using it for BCI studies or for other research. Support mechanisms, including a BCI2000 newsletter and an online bulletin board system have been initiated and are functioning very well.

ISSUES

Work is clearly going very well. The most critical issues continue to concern how to allot the Partnership's time and effort among the many exciting opportunities now presenting themselves.

In addition to the active projects described above, we are pursuing additional ECoG-related studies with Dr. Ojemann at the University of Washington in Seattle and Dr. Koszer at Albany Medical College. We are also further developing the P300-related clinical collaboration with the BRP consultant Dr. Donchin in Tampa. These initiatives are likely to be very productive. At the same time, they make substantial demands on BRP personnel.

A steadily growing number of research groups are using BCI2000. These groups vary greatly in their expertise. Some are able to use BCI2000 with minimal consultation, while others need much help, often because they lack essential expertise not directly related to BCI2000. We continue to struggle with defining and implementing reasonable criteria for giving this help and reasonable limitations on the time and effort we allow it to occupy. We are also receiving requests from people wanting to come to the Wadsworth Center to learn to use BCI2000 and BCI research techniques in general. We believe that a three-week course to be given periodically, perhaps once a year, would be effective and efficient. At the same time, this would be a major commitment that we do not at present have the time or resources to undertake. A grant proposal to fund this project may be indicated.

PI: John Wong, Ph.D.
William Beaumont Hospital
Department of Radiation Oncology
3601 West Thirteen Mile Rd
Royal Oak, MI 48073
T: 248-551-6583
F: 248-551-3784
jwong@beaumont.edu

PROJECT TITLE: An image guided small animal radiation research platform

PARTNERS' NAMES AND AFFILIATIONS:
Elwood Armour, Ph.D., Qiuwen Wu, Ph.D. (William Beaumont Hospital)
Randy Gu, Ph.D. (Oakland University, Auburn Hills, MI)
Licai Jiang (Osmic Inc., Auburn Hills, MI)

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

ABSTRACT
Significant advances have been made in medical anatomical imaging over the past 25 years. More recently, there has been increased interest in small animal biological imaging research based on the increasing understanding of various molecular and genetic signals in tumor cells in vitro and in vivo. These initiatives have focused primarily on therapeutic research with drugs and other systemic agents, somewhat ironically, overlooking radiation therapy. The addition of biological imaging information to radiation therapy will have a major impact on the management and evaluation of the treatment. Unfortunately, despite the interest in biological imaging research, animal radiation research methodology lags far behind clinical practices. Advanced techniques such as conformal, intensity modulated radiation therapy (IMRT) is increasingly becoming routine in human clinics, and has led to a shift in the clinical paradigm of the uniform dose delivery towards non-uniform dose delivery, particularly to the critical organs. The advent of new image guidance methods for short course radiation treatment will yet lead to the delivery of dose distributions of greater non-uniformity. Regrettably, present laboratory research equipment prohibits testing of these paradigms in animal models. As a consequence, the advanced treatment technologies are applied clinically without any guidance from small animal radiation experimentation to evaluate efficacy in a preclinical setting. In this proposal we request funding for the de novo construction, testing and evaluation of an image guided small animal radiation research platform (SARRP) that will accurately deliver complex ionizing radiation dose distributions in small animal tumor model systems, mice, rats and rabbits. Specifically we propose 1) the construction of a gantry system containing three kilovoltage (kV) radiation sources that will have energy deposition resolution of ≈1mm and on-board cone beam CT imaging resolution of ≈0.5mm; 2) the development of dosimetric and treatment planning methodologies that parallel that for human treatment. Finally (3) the third specific aim is to develop methods of precise animal setups and to validate the imaging and irradiation capabilities of the system for accurate delivery of localized dose distributions in small animals. The research requires integration of expertise in mechanical engineering, x-ray optics and radiation dosimetry physics. The collaborative efforts are best coordinated in a Bioengineering Research Partnership (BRP). An Oversight Group is formed to evaluate the progress of the BRP, and to help identify opportunities and hypotheses for future research.

STATUS OF RESEARCH AND PARTNERSHIP

We have strengthened the BRP with the inclusion of Analogic, a major manufacturer of imaging systems for medical and security applications. They are providing us with a slip-ring gantry from which to mount the imaging and irradiation components. The effort will be conducted at Oakland University. A source to isocenter distance of 45 cm has been finalized. Based on this geometry, Osmic has begun lens design and construction. Concurrently, at Beaumont, the alpha-version of Monte Carlo calculations in the Phillips/ADAC Pinnacle planning system is under evaluation for kilovoltage calculations. Experiments are being conducted using radiochromic and silver halide films for absolute and relative dosimetry measurements, respectively.

To maintain cohesiveness of research, the Principal Investigator group meets every week and the BRP meets every 2 weeks. The Groove software has been installed for all members of the BRP to communicate progress and issues.

A combined workshop of Oncologic Target Imaging and Small Animal Radiation Research is planned for July 23-24 which includes international and national investigators. The oversight group will also participate.

ISSUES

The mandatory 18% budget reduction hurts our effort, particularly for our small budget relative to other BRP grants. We may not be able to implement a 3-source system which will result in working at a less than optimum dose rate for animal experiments.

PI: Ajit P. Yoganathan, Ph.D.
Georgia Institute of Technology
Wallace H. Coulter School of Biomedical Engineering
313 Ferst Drive
Atlanta, GA 30332
T: 404-894-2849
F: 404-385-5013
ajit.yoganathan@bme.gatech.edu
www.bme.gatech.edu/groups/cfmg/cfmg.htm

PROJECT TITLE: Understanding / Improving Flow Dynamics in Fontan Surgeries

PARTNERS' NAMES AND AFFILIATIONS:
Mark A. Fogel, M.D. (Children's Hospital of Philadelphia), Carol Lucas, Ph.D. (University of North Carolina), W. James Parks, M.D. (Emory University School of Medicine, Children's Healthcare of Atlanta, Sibley Heart Center), Shiva Sharma, M.D. (Pediatric Cardiology Associates)

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

ABSTRACT
The overarching hypothesis is that a fundamental understanding of the single ventricle and associated total cavopulmonary (TCPC) anatomy and physiology will lead to improved surgical planning and designs and thus the potential for improved long term outcomes for patients. In order to address this, we plan to investigate the following specific aims: (1): Qualitatively and quantitatively assess Fontan flow dynamics for different TCPC anatomic geometries and physiologic conditions in order to establish optimal TCPC anatomic templates, (2): Study the impact of different materials, used in the IVC to RPA connection (in the TCPC surgery), on the local flow dynamics, (3) Establish an anatomic and materials database that would be used to validate computationally based surgical planning and designs, (4): Provide improved surgical planning and designs through the use of pre surgery MRI anatomic information and computational simulations to optimize the TCPC in an individual patient, (5): Study the effects of ascending aorta flow dynamics on the energetics of the Fontan connection, (6): Explore the feasibility of using a "pressure regulator/pump" to help reduce central venous pressure in Fontan patients and thus improve their long term outcome and quality of life.

STATUS OF RESEARCH AND PARTNERSHIP
Studies at Georgia Tech focused primarily on the development and validation of the computational (CFD) and experimental fluid dynamics analysis methodology. An anatomic case was studied in detail as its flow field appeared very complex, involving flow instabilities and transitions from laminar to turbulent regimes. In vitro computational and experimental studies revealed that even though the caval inflow conditions are specified as steady, flow within the connection region was transient. Three more anatomic models are currently being analyzed through this validated CFD methodology. Using state-of-the-art surface manipulation tools and computer-aided design codes, we have streamlined the process in going from magnetic resonance imaging (MRI) reconstruction to a CFD grid and an in vitro stereolithography model.
A transparent anatomic rapid prototype model production protocol for in vitro digital particle image velocimetry (DPIV) experiments is finalized. Validation of the methodology is completed by comparing the experimental results with the earlier experiments that employed idealized glass models. The differences in measured hydrodynamic control volume power losses were not significant in the laminar regime ($p>0.1$ at 2 and 4L/min, which are the normal and exercise operating cardiac outputs in Fontan children), but were at 6L/min ($p<0.05$) where the maximum Reynolds number is 2023. These anatomical models are selected so that we have at least one comprehensive experimental/computational study for each template.
At the University of North Carolina at Chapel Hill (UNC), significant progress has been made in Year 2 in two areas: ex vivo studies on the hemodynamics of right heart bypass operations using the explanted hearts of post surgery lambs and in vivo animal studies.

The Children's Healthcare of Atlanta (CHOA), the Children's Hospital of Philadelphia (CHOP), and UNC each transferred MRI patient data sets to a secure server at Georgia Tech. Currently; the patient database contains MRI (anatomy and flow) information on 60 TCPC patients. The mean age is 10.3 years and there is a 2:1 ratio of males to females. Most patients are non-Hispanic Caucasians, and intra-atrial connections dominate the patient population. Within the data sets all five templates of the TCPC are represented. However, the interrupted IVC and the IVC-MPA connection were limited because of very few cases of heterotaxy syndrome and because only one surgeon performed the IVC-MPA connection.
Axial MR anatomy images are obtained via balanced fast gradient echo sequences. In order to create data sets composed of isotropic voxels, which are better suited for reconstruction, a technique called adaptive control grid interpolation (ACGI) is used to enhance through-plane spatial resolution. Each TCPC is isolated within the enhanced MR data using a shape-element segmentation technique. Intensity thresholding and edge detection methods are used to create a scaffold around the TCPC, within which the vascular area of interest is defined by the motion of a shape element. Computer-aided design tools are used to produce a 3-D model of the TCPC from the segmented data to be used in rapid prototyping and CFD.
Our evaluation of the reconstructed TCPC pathways demonstrated the finding of long segment LPA stenosis in 13 of 37 cases (35.1%). In this group there were 3 cases from CHOA and 10 cases from CHOP. Further analysis revealed that 10 of the 13 cases (76.9%) were patients with hypoplastic left heart syndrome. Analysis of the axial and sagittal plane MR images revealed that the LPA stenosis was due to anterior and superior compression from a dilated neo-ascending aorta.
In Matlab, a program called Flowfinder was written to analyze the velocity images. The program calculates mean flow for the entire cardiac cycle, mean flow for each phase in the cardiac cycle, maximum velocity in each phase of the cardiac cycle, mean velocity during the cardiac cycle, and area of the vessel lumen. The velocities extracted by Flowfinder can then be used as boundary conditions for the CFD simulations.

ISSUES
At the beginning of the project, the complexity of flow physics was unknown. Because of such detailed flow fields, in-house CFD solvers are now required for good experimental agreement. Commercial codes and lower-order steady CFD analysis appeared to be useful only for gross hemodynamic performance indices such as energy loss. Additionally, the small size of the TCPC gave rise to a large dependence on the inflow boundary conditions. These findings, being unique in the TCPC literature, have been submitted for publication. While perfect agreement with experimental flow fields was accomplished, the transient higher-order CFD solutions required massive computational resources (almost 200 fold increase in the CPU time). These difficulties and the consequent unforeseen extra validation effort, has reduced the number of completed anatomical CFD solutions. Accordingly to address these computational requirements, in the future, a multi node high-performance computer is planned to be operational by July 2004.
The complex three-dimensional flow fields encountered in TCPC anatomies have also accelerated our DPIV system upgrade efforts. The new system, which has been installed and calibrated, will increase both the spatial and temporal resolution of the experimental data. The acquisition of large data sets gives us the capacity to perform frequency analysis of the unsteady complex flow phenomena that is observed in the studied Fontan geometries. The system includes a stereo component allowing planar measurement of all three components of velocity and thus the potential to reconstruct high-resolution three-dimensional velocity fields from the TCPC.

The investigators and research staff at CHOA, CHOP, and UNC will continue to work diligently to enroll minority and female subjects into the above referenced study. We are, however, very cognizant of the fact that our patient population is, for the most part, white males.

Though the initial aim of the reconstruction database was not to generate a detailed clinical study, the quality, volume of data, and 3D visual access to the anatomic information have incited several interesting studies. One particular observation that we plan to report on next year is related to long segment LPA stenosis. To this end, we plan to integrate contributions of pediatric cardiothoracic surgeons to highlight the clinical impact of our studies.

PI: Yan Yu, Ph.D., MBA
University of Rochester Medical Center
Department of Radiation Oncology
601 Elmwood Avenue, Box 647
Rochester, New York 14642
T: 585.275.9988
F: 585.275.1531
Yan_Yu@urmc.rochester.edu

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

ABSTRACT
Intratumoral therapies of prostate cancer include the delivery of brachytherapy or ablation energy sources, and adenoviral injection, through a minimally invasive, transperineal approach. They require quantitative, optimized treatment planning, precise placement of the needles/probes according to the treatment plan, and real time dosimetric evaluation in the operating room as deviations from the treatment plan are detected. Recent studies suggest that imprecisions in standard brachytherapy using the manual technique cause higher than previously-appreciated complication rates, and may be the cause of local failure in 15% of the patients.

The major goal of the proposed work is to develop the Robot-Assisted Platform for Intratumoral Delivery (RAPID) for integrated treatment of prostate cancer, and to demonstrate the safety, efficacy and clinical effectiveness through bench/phantom tests and a Phase I clinical study. A number of maturing component technologies previously developed by the bioengineering research partners will be combined in this major collaboration, including the first robotic system for urological applications with early experience on actual patient treatment, the first treatment planning system with intraoperative dosimetry optimization, and the first needle/probe tracking system for real time ultrasound-based treatment verification, permitting re-planning and re-optimization of therapy delivery. The integrated RAPID system, designed based on prototype subsystems developed at each of the research partners, will initially focus on interstitial brachytherapy of prostate cancer. It is aimed at delivering precise, non-coplanar 3D conformal radiation rapidly and with assured consistency, and to incorporate such complex concurrent therapies as radiosensitization and mixed agent/strengths brachytherapy. Primary outcome variables including implant quality, cost, morbidity and learning curve will be examined under the clinical study by comparison with historical controls.

The long-term objective of the RAPID project is to incorporate the delivery of concomitant therapeutic agents intratumorally for cancer in the prostate as well as in other organ systems. The multi-agent, multi-modality capabilities of the RAPID system will be continually exploited towards total optimization of a turnkey in vivo diagnosis and therapy engine for localized cancers of solid organs.

STATUS OF RESEARCH AND PARTNERSHIP

At this time the project is in the 10th months and is progressing well. In the first 6 months, we devoted a significant portion of efforts to evaluating the feasibility of a novel, conical approach to interstitial needle access. Conventional interstitial therapies for prostate, gynecological and breast cancers all employ a rectilinear approach via template guidance, in which needles by intention are all parallel to one another. In contrast, the proposed conical implant approach uses an isocentric technique for placing needles; needles are projected into tissue from a pivot point, which may be placed either at the skin surface through a sheath to prevent multiple punctures, or at depth to overcome anatomical constraints. Inverse planning algorithms were developed to optimize the angulation pattern of the needles and the spacing of therapeutic sources based on retrospective analysis of 20 unselected patients who underwent prostate brachytherapy. This study demonstrated dosimetric feasibility of conical implantation of low energy radioactive sources to the prostate. Work is now ongoing to implement a hybrid system capable of rectilinear, angulated and conical approaches.

For the robotic platform, we have completed requirement gathering and preliminary conceptual design of the main functional subsystems. High-level design and detailed design should be completed by mid Yr 2, followed by 3 months of fabrication and unit testing. At the same time, a testbed robotic system is being assembled to investigate key issues in the final design.

Software and control system requirements have been formalized following software engineering principles, and an overall design generated. The software platform incorporates real-time automated image guidance and augmented reality, feedback control, active steering, as well as dynamic dosimetry. UML diagrams and C++ codes will be developed in the next phase.

A high-order finite element formalism was implemented to model organ deformation using a small number of cubic-Hermite elements. The cubic-Hermite formalism allows for curvilinear element boundaries and non-linear interpolation of deformation within each element. Prostate surface feature points extracted from serial organ slices were fit to 6- and 8- element FEM models and subjected to pin-cushion deformations using a linear-elastic model of tissue mechanics. The force of deformation was adjusted to recreate an overall surface displacement analogous to that observed clinically. In the next phase of development of the model we will determine and incorporate more realistic non-linear mechanical behavior of the tissue. The FEM model will then allow us to access the forces exerted by the needle on the prostate and predict and correct for tissue deformation in the placement of therapeutic sources.

ISSUES

There is no major issue at this time. Weekly progress meetings at the principal BRP institution and bi-weekly net-meetings amongst the BRP institutions appear quite effective. This is complemented by mutual visits of 7 different investigators (partially supported by the grant). At the start of the grant period, a key investigator on the software side (Dr. Fei) became interested in a faculty position at the principal BRP institution. This would be a positive development, as it permits synergistic consolidation of expertise. However, administrative paperwork for the faculty appointment took 7 months to complete, which caused some minor delays. We have adjusted the project schedule and personnel efforts to ensure that progress will be back on track by the end of grant Yr 2.