Surfaces in Biomaterials Foundation

BioInterface 2005

Symposium Notebook

October 24 - 26, 2005
The Marquette Hotel • Minneapolis, MN

Program Chair, Mark Moore

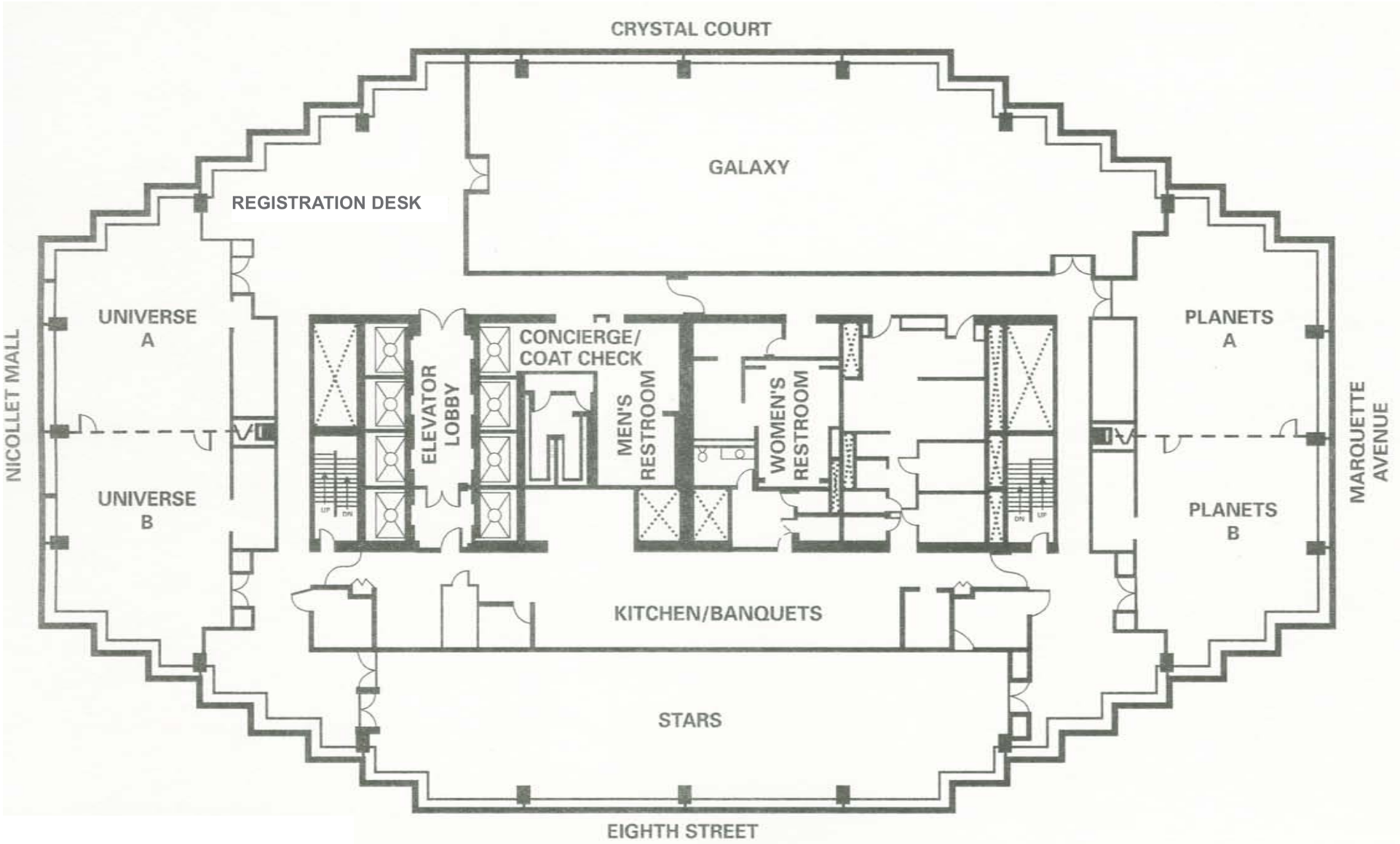

TABLE OF CONTENTS

SESSION ABSTRACTS

INTRODUCTION

BIOINTERFACE 2005

This year, the Surfaces in Biomaterials Foundation celebrates its 15 year anniversary with special guests and events at BioInterface 2005. The venue of Minneapolis is very fitting given the breadth of medical technology and bioscience industries in the Twin Cities area.

The Surfaces in Biomaterials Foundation is dedicated to exploring creative solutions to technical challenges at the BioInterface by fostering education and multidisciplinary cooperation among industrial, academic, clinical and regulatory communities.

ABOUT THE FOUNDATION

The Surfaces in Biomaterials Foundation is dedicated to exploring creative solutions to technical challenges at the BioInterface by fostering education and multidisciplinary cooperation among industrial, academic, clinical, and regulatory communities.

The Surfaces in Biomaterials Foundation provides a forum for the presentation of contributions to surface science. The Foundation's Academic and Supporting Members explore surface characterization and problem-solving techniques important in the manufacturing, development, and research of biomaterials and offer a strong, united voice within the community.

OUR MISSION

The Surfaces in Biomaterials Foundation is dedicated to exploring creative solutions to technical challenges at the BioInterface by fostering education and multidisciplinary cooperation among industrial, academic, clinical and regulatory communities.

CONTACT THE FOUNDATION

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ACKNOWLEDGEMENTS

The Surfaces in Biomaterials Foundation would like to thank the following individuals and companies for making this program possible:

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SCHEDULE AT-A-GLANCE

MONDAY, OCTOBER 24

5:00 - 6:00 p.m.	Applied Technology Workshop (Universe A & B)
6:00 - 7:30 p.m.	Opening Reception and Keynote (Universe B)

TUESDAY, OCTOBER 25

7:30 - 9:00 a.m.	Registration and Continental Breakfast (Galaxy Room)
7:30 a.m. - 6:00 p.m.	Student Poster Session (Stars Room)
7:30 - 8:45 a.m.	Student Poster Session Judging (Stars Room)
9:00 - 9:15 a.m.	Welcome (Galaxy Room)
9:15 - 10:45 a.m.	BIOADHESION (Galaxy Room)
10:45 - 11:15 a.m.	Break and Exhibits (Stars Room)
11:15 - 11:45 a.m.	HEALING OF VASCULAR DEVICES (Galaxy Room)
11:45 a.m. - 12:30 p.m.	Business Meeting (Galaxy Room)
12:30 - 1:30 p.m.	Student Town Hall (Universe A & B)
12:30 - 1:30 p.m.	Lunch and Exchibits (Universe A & B)
1:30 - 3:00 p.m.	PERIPHERAL VASCULAR DISEASE TREATMENTS (Galaxy Room)
3:00 - 3:30 p.m.	Break and Exhibits (Stars Room)
3:30 - 5:30	RUMP SESSION (Galaxy Room)

WEDNESDAY, OCTOBER 26

8:00 a.m. - 3:30 p.m.	Poster Session (Stars Room)
8:00 - 9:00 a.m.	Continental Breakfast (Galaxy Room)
9:00 - 10:30 a.m.	MATERIAL CHARACTERIZATION (Galaxy Room)
10:30 - 11:00 a.m.	Break and Exhibits (Stars Room)
11:00 a.m. - 12:15 p.m.	INVENTION SYMPOSIUM (Galaxy Room)
12:15 - 1:30 p.m.	Lunch and Awards Session (Universe A & B)
1:30 - 2:00 p.m.	Break and Exhibits (Stars Room)
2:00 - 3:30 p.m.	HUMAN CELLULAR AND TISSUE-BASED THERAPY (Galaxy Room)

SYMPOSIUM SCHEDULE

MONDAY, OCTOBER 24

5:00 - 6:00 p.m. *Asylum Research*	**Applied Technology Workshop** Atomic Force Microscopy Using the MFP-3D AFM (Universe A)
Plasma Technology Systems, LLC	Diversity of Plasma Applications for Biomaterials (Universe B)
6:00 - 7:30 p.m.	**Opening Reception and Keynote** (Stars & Universe B)
6:30 - 7:00 p.m.	**Keynote Speaker: Acheiving and Verifying Tissue Adherence to Assure Performance of Hydrogel-Based Medical Devices** ***Art Coury, Genzyme Corporation***

TUESDAY, OCTOBER 25

7:30 - 9:00 a.m.	Registration & Continental Breakfast (Galaxy)
7:30 a.m. - 6:00 p.m.	Student Poster Session (Stars)
7:30 - 8:45 a.m.	Student Poster Session Judging (Stars)
9:00 - 9:15 a.m.	Welcome (Galaxy) *Robert Elde, Dean, College of Biological Sciences, University of Minnesota*

Bioadhesion

9:15 - 9:45 a.m.	**Theories or Mechanisms of Adhesion** (Galaxy) ***K.L. Mittal, J. Adhesion Sci. Technol.***
9:45 - 10:05 a.m.	Differentiation of Cells and Stem Cells with Surface-bound Photo Reagents *Muhammad Lodhi, SurModics, Inc.*
10:05 - 10:25 a.m.	Basic Considerations for Adhesion to Skin *Donald Lucast, 3M Medical Division*
10:25 - 10:45 a.m.	Thermally Reactive Polymers for Medical Device Coatings *Kristin S. Taton, Innovative Surface Technologies, LLC*
10:45 - 11:15 a.m.	BREAK and EXHIBITS (Stars)

Healing of Vascular Devices

11:15 - 11:45 a.m.	***Nicolas Chronos, American Cardiovascular Research Institute*** (Galaxy)
11:45 a.m. - 12:30 p.m.	Business Meeting (all invited) (Galaxy)
12:30 - 1:30 p.m.	Student Town Hall (with lunch) (Universe A & B)
12:30 - 1:30 p.m.	LUNCH and EXHIBITS (Universe A & B, Stars)

Peripheral Vascular Disease Treatments

1:30 - 2:00 p.m.	**Novel Protein Therapies for Vascular Tissue Engineering** (Galaxy) ***Eric Brey, Illinois Institute of Technology***
2:00 - 2:20 p.m.	Surface Chemistry, Pre-Clinical and Clinical Results of a Thromboresistant Heparin-Immobilized ePTFE Vascular Graft *John Fisher, WL Gore & Associates*
2:20 - 2:40 p.m.	Membrane-Mimetic Films Containing Thrombomodulin and Heparin Inhibit Tissue Factor-Induced Thrombin Generation in a Flow Model *Elliot Chaikof, Emory University*

SYMPOSIUM SCHEDULE *(cont)*

2:40 - 3:00 p.m. Fabrication of Hollow Alginate Fibers for Cellular Encapsulation
Philip Brown, Clemson University

3:00 - 3:30 p.m. BREAK and EXHIBITS (Stars)

Rump Session

3:30 - 5:30 p.m. Who Rules the Hospital: Cardiologist vs. Cardiac Surgeon (Galaxy)
Robert S. Schwartz, MD, FACC, FAHA, Minnesota Cardiovascular Research Institute, Minneapolis Heart Inst Foundation Minneapolis
Eric Brey, Illinois Institute of Technology

WEDNESDAY, OCTOBER 26

8:00 a.m. - 3:30 p.m. Poster Session (Stars)
8:00 - 9:00 a.m. Continental Breakfast

Material Characterization

9:00 - 9:30 a.m. **Microscopy of Soft, Conductive, and Bioactive Materials for Interfacing Electronic Devices with Living Tissue** (Galaxy)
David Martin, University of Michigan

9:30 - 9:50 a.m. Morphology of Polymer Blend Coatings for Drug Delivery
Klaus Wormuth, SurModics, Inc.

9:50 - 10:10 a.m. Evaluation of Acrylate-Based Block Copolymers Prepared by Atom Transfer Radical Polymerization as Matrices for Paclitaxel Delivery from Coronary Stents
Robert Richard, Boston Scientific Corporation

10:10 - 10:30 a.m. Human Platelet Responses to Silicon-Alloyed Pyrolytic Carbons
Steve Goodman, 10H Inc.

10:30 - 11:00 a.m. BREAK and EXHIBITS (Stars)

Invention Symposium

11:00 a.m. - 12:15 p.m. Special Lecture (Galaxy)
Joachim Kohn, New Jersey Medical School, Rutgers University

12:15 - 1:30 p.m. LUNCH and Awards Session (Universe A & B)
1:30 - 2:00 p.m. BREAK and EXHIBITS (Stars)

Human Cellular and Tissue-Based Therapies

2:00 - 2:30 p.m. **Adult Derived Mesenchymal Stem Cells and Potential Clinical Applications** (Galaxy)
Russel Reiss, VA Hospital, University of Utah School of Medicine

2:30 - 2:50 p.m. Comparison of Bone Marrow and Blood Mononuclear Cell-Seeded Biologocial Matrices for Myocardial Tissue Repair
Jeremy Ollerenshaw, American Cardiovascular Research Institute

2:50 - 3:10 p.m. Engineering a Biohospitable Acellular Cardiovascular Tissue
Alyce Linthurst Jones, LifeNet

3:10 - 3:30 p.m. The Use of Adult Derived Adipose Stem Cells for Bone Regeneration
Kelly Kirker, Bacterin

AWARD WINNER

EXCELLENCE IN SURFACE SCIENCE AWARD

"Progress Towards an Anticoagulant Independent Mechanical Heart Valve"

Dr. Jack Bokros
MCRI

This award honors outstanding researchers for significant contributions to the field of surface science. This year's awardee is Dr. Jack Bokros of MCRI. Through Dr. Bokros' efforts to proliferate the use of pyrolitic carbon through education and working with numerous partners, the material has become ubiquitous in mechanical heart valve applications. His impact on medical device technology goes far beyond mechanical heart valves. As the President of Carbomedics, Dr. Bokros established Intermedics Orthopedics Incorporated and Calcitek Incorporated to commercialize porous metal, calcium hydroxyapatite and carbon biomaterial technologies that encouraged and secured bone ingrowth. The application of pyrolitic carbon technology for orthopedics devices proved more challenging but MCRI is now producing pyrolitic carbon coatings for small joints, which are able to restore joint function that was not possible with polymer and metal devices. He has also authored more than 100 publications and has become a named inventor on more than 50 patents. Dr. Bokros' devices and materials have impacted more than two million patients since the first pyrolitic carbon heart valve was implanted 37 years ago.

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KEYNOTE SPEAKER

Achieving and Verifying Tissue Adherence to Assure Performance of Hydrogel-Based Medical Devices

Arthur J. Coury
Genzyme Corporation
Cambridge, MA

Monday, October 24, 2005
6:30 - 7:00 p.m.

Invited Speaker

ACHIEVING AND VERIFYING TISSUE ADHERENCE TO ASSURE PERFORMANCE OF HYDROGEL-BASED MEDICAL DEVICES

Arthur J. Coury, Genzyme Corporation
Cambridge, MA

Durable, programmable adherence to tissue is warranted in hydrogel-based medical devices such as surgical sealants, tissue adhesives, skin electrodes, wound dressings and drug delivery systems. Appropriate tissue bonds can be achieved by several mechanisms. When fluid flow enables penetration of the adhesive into tissue interstices, mechanical "interlock" occurs. Pressure sensitive adhesives (which are fluids by definition) as well as in situ polymerized hydrogel forming compositions may accomplish this. Secondary hydrogel-tissue bonding interactions are based on coulombic and VanDer Waals forces and occur with intimate contact of the hydrogel with the tissue. In our sealants, we use a very effective proprietary in situ bonding process employing a reactive primer over which a topcoat is chemically bonded. This exploits both the mechanical interlock and the secondary bonding forces. Desiccated hydrogels provide such contact by swelling on moist tissue surfaces. Systems undergoing liquid to solid transformations as described above attain some of their bonding by this process. The most durable bonds are formed by covalent coupling of the hydrogel to the tissue which occurs by in situ chemical reaction. In some two part adhesives and sealants, one of the components reacts covalently with tissue as well as with the other component.

Insights into the mechanisms and magnitudes of hydrogel-tissue bonding may be gained using mechanical bonding and visualization tests. To estimate mechanical strength of hydrogel-tissue bonds, we have adapted several of the testing methods of coatings and adhesives scientists. In addition, we have developed tissue-specific adhesion tests. Shear, peel and butt tensile specimens, aged in vitro in appropriate solutions provide useful quantitative values for initial and aged bond strengths. In a burst test, a tissue film with a defect is mounted in a jig, coated, hydraulic or pneumatic pressures are applied to failure and values are registered. Cyclic strain tests of hydrogel coatings over tissues such as lung pleura, pericardium and dura mater are amenable to subjective evaluation using postaging "pick tests" by an experienced evaluator. We have also developed a needle pull-through test to determine bond strength of tough hydrogels to orthopedic tissues.

However appropriate the in vitro tests, performance of tissue adherent devices, especially implanted ones, must generally be studied by preclinical in vivo tests prior to clinical studies. Imaging histology slides from in vivo implants visualizes hydrogel-cell interactions including bonding characteristics, which can be followed over time. Scanning electron microscopy of explants is also valuable for producing magnified images of surface topography and hydrogel-tissue interfaces. With both the foregoing techniques, effects of desiccation on dimensions during sample preparation must be considered.

Ultimately, adhesive performance of hydrogel devices must be verified in the clinic. Adherence testing on intact skin presents a minimal regulatory hurdle. For a skin electrode product, we employed a sauna-based test in which we were able to characterize human subjects as supporting high, medium and low adherence and qualified the product based on efficacy on the "low stickers." For implantable adhesives, clinical studies are more complex, occurring in phases and only rarely amenable to "second look" surgeries. In such cases, efficacy is studied by evaluating symptomatic relief of the pathologic conditions.

By studying bonding mechanisms and applying effective bonding techniques, we have developed several commercial hydrogel products as adhesion prevention barriers, surgical sealants and electrodes.

Additional studies on tissue adhesives and space fillers, which employ specific bonding tests, are underway.

BIOGRAPHY OF INVITED SPEAKER

ARTHUR J. COURY

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Art Coury holds a B.S. degree in chemistry from the University of Delaware (1962), a Ph.D. in organic chemistry (1965) and an M.B.A. (1980) from the University of Minnesota. His industrial career includes positions as Senior Research Chemist at General Mills, Inc. (1965-1976), as Director, Polymer Technology and Fellow at Medtronic, Inc. (1976-1993), as Vice President, Research and Chief Scientific Officer at Focal, Inc. (1993-2000), and as Vice President, Biomaterials Research at Genzyme Corporation (2000-Present).

His career focus has been polymeric biomaterials for medical products such as implantable electronic devices, hydrogel-based devices and drug delivery systems. He holds 50 distinct patents and has published and presented widely in his field. His teaching positions have included adjunct appointments at the University of Minnesota and the Harvard-MIT Graduate Program in Health Sciences and Technology.

His public service positions have included: Chair, Minnesota Section, American Chemical Society (1989-1990); President, Society for Biomaterials, USA (1999-2000); President, American Institute for Medical and Biological Engineering (2003-2004) and membership on several university technical advisory boards.

BIOADHESION

Tuesday, October 25, 2005

9:15 - 10:45 a.m.

9:15 - 9:45 a.m. **INVITED SPEAKER**
Theories or Mechanisms of Adhesion
K.L. Mittal, Journal of Adhesion Science and Technology

9:45 - 10:05 a.m. Differentiation of Cells and Stem Cells with Surface-bound Photo Reagents
Muhammad Lohdi, SurModics, Inc.

10:05 - 10:25 a.m. Basic Considerations for Adhesion to Skin
Donald Lucast, 3M Medical Division

10:25 - 10:45 a.m. Thermally Reactive Polymers for Medical Device Coatings
Kristin S. Taton, Innovative Surface Technologies, LLC

Invited Speaker

THEORIES OR MECHANISMS OF ADHESION

K.L. Mittal, Editor, Journal of Adhesion Science and Technology
Hopewell Junction, NY

Many theories or mechanisms of adhesion have been proposed to explain adhesion between similar or dissimilar materials. These include: mechanical interlocking, adsorption, surface energetics or wettability, diffusion, electrostatic, chemical, acid-base, and weak boundary layer. Examples, ranging from adhesive bonding to metallized plastics, will be cited to show the relevance of these theories, and their limitations will be highlighted. There is no universal theory of adhesion which can explain all observed adhesion behaviors. Apropos, the concept of the weak boundary layers is a way to explain lack of adhesion. Examples of how WBLs are created and how to eliminate them will be given.

In the last decade or so, the concept of acid-base interactions has evinced considerable interest in the realm of adhesion so in this talk the acid-base theory of adhesion will be accorded due coverage.

BIOGRAPHY OF INVITED SPEAKER

K.L. MITTAL

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Dr. Kashmiri Lal Mittal was associated with the IBM corporation from 1972 through 1993. He is currently teaching and consulting worldwide in the areas of adhesion and surface cleaning. He has initiated, organized and chaired a large number of international symposia and is the editor of 79 published books and others that are currently in print dealing, inter alia, with adhesion measurement, adhesion of polymeric coatings, polymer surfaces, adhesive joints, adhesion promoters, thin films, polyimides, surface modification, surface cleaning and surfactants. Dr. Mittal has received many awards and honors including the 1990 Dudley Award of the ASTM, the 1995 Thomas D. Callinan Award of the Electrochemical Society, the 1997 John A. Wagnon Technical Achievement Award of the IMAPS, the Adhesives Age Award, is a Robert L. Patrick Fellow member of the Adhesion Society and is listed in many biographical reference works. He is the Editor of the International Journal of Adhesion Science and Technology, and is/has been a member of the editorial boards of a number of scientific and technical journals. Dr. Mittal was recognized for his contributions and accomplishments by the worldwide adhesion community, who organized, in his honor, the 1st International Congress on Adhesion Science and Technology in Amsterdam in 1995. In 2002, he was honored by the global surfactant community by the inauguration of the Kash Mittal Award, sponsored by the Nestle Research Center in Lausanne, Switzerland, in the surfactant field for his vast efforts and significant contribution to the field of colloid and interface chemistry. In October 2003, he was honored by the Maria Curie-Sklodowska University, Lublin, Poland with the awarded title of Doctor Honoris Causa.

DIFFERENTIATION OF CELLS AND STEM CELLS WITH SURFACE-BOUND PHOTO REAGENTS

Tahmina Naqvi, Gary Opperman and Muhammad Lodhi
SurModics, Inc., 9924 West 74th St., Eden Prairie, MN 55344
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Cell differentiation is a highly controlled process whereby undifferentiated cells are induced to perform more elaborate function or make specialized cells, tissues and organs. Cell differentiation, like adhesion and proliferation, takes place on extra cellular matrix, which is an intricate network of several proteins and glycoproteins, including collagen, and proteoglycans and provides the architecture and support to tissues in vivo. Some of the ECM proteins, such as collagen, fibronectin and laminin are used in vitro to mimic the intrinsic environment but are limited due to several functional and regulatory reasons. Current commercially available synthetic materials fail to produce reliable differentiation of cells.

We have developed photo polymers for cell attachment, proliferation and differentiation of rat PC12 cells as well as mouse embryonic stem cells, ES-D3. Comparative data has been generated on photopolymers and three commercial products, viz., Synthetic ECM™, PuraMatrix™ and Matrigel™ that are designed to achieve three dimensional extra cellular matrix like structure. Quantitatively better cell differentiation has been achieved in PC12 cells grown on substrates coated with photo reagents. In addition, ES-D3 stem cells attach and divide well on coated surfaces as well as remain totipotent in the presence of FGF. With proper change in the growth conditions, cells differentiated into neurons, oligodendrocytes and type I and type II astrocytes. The new surface has been analyzed with standard surface characterization techniques such as SEM and AFM. Data will be presented.

Keywords: Photo polymers, cell differentiation, cell attachment, PC12, ES-D3 stem cells, ECM.

BASIC CONSIDERATIONS FOR ADHESION TO SKIN

***Donald Lucast*, 3M Medical Division**
dhlucast1@mmm.com

Adhering medical devices such as tapes and wound dressings to skin presents many challenges. From the earliest patent on a pressure-sensitive adhesive (PSA) to the current range of synthetic polymer PSAs, both materials and device constructions have been improved and refined to optimize performance in specific applications. Natural rubber and synthetic polymers such as acrylates, block copolymers, silicones, and other specialty polymers have been used successfully on medical devices. Skin presents many problems as a substrate for adhesive devices. It is weak, rough, contaminated with salts and oils, and has low surface energy. It varies with age, gender, ethnicity, diet, and environment. In addition, special challenges arise from wet, damaged, or fragile skin.

THERMALLY REACTIVE POLYMERS FOR MEDICAL DEVICE COATINGS

***Kristin S. Taton**, Christina K. Thomas, William B. Knopke,*
Timothy A. Bloomquist, John V. Wall, Jie Wen, and Patrick E. Guire
Innovative Surface Technologies, LLC, 1000 Westgate Dr., Suite 115, St. Paul, MN 55114
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Current technology for coating medical devices usually involves multiple coating steps in various solvents, plasma activation, photoactivation, or a combination. In many cases, a medical device is incompatible with organic solvents and/or high temperatures or is incompatible with line-of-site coating required for surface activation. We have developed a new one step coating method using mild heating conditions (<80°C) in aqueous or alcoholic solvents. The thermally reactive polymer is a copolymer composed of most common lubricious or passivating polymers such as poly(vinylpyrrolidone), poly(acrylamide) or poly(ethylene glycol), with thermally reactive perester groups. The perester group decomposes thermally under mild heating to produced two radicals, which can then crosslink the coating polymer to the medical device substrate surface. The thermally reactive polymers have been characterized by NMR and FTIR to show significant crosslinking occurs within the two hours of heating.

Figure 1 shows the NMR decomposition of perester at 80°C with the decrease of the perester peak, and growth of peaks due to acetone and tert-butanol, two common decomposition products. Similar FTIR experiments show the loss of perester peak (1769 cm^{-1}) as heating occurs. This crosslinking has been shown to bond coatings to common medical device substrates such as poly(propylene), PBAX, poly(urethane), poly(imide), and pre-treated stainless steel. The coatings have been evaluated by contact angle, SEM, protein adsorption assays, and friction testing. Such coatings reduce the non-specific adsorption of proteins *in vitro* by 80% or better. The thermally reactive copolymer coatings developed should enhance medical device function on those devices where traditional coatings methods are ineffective.

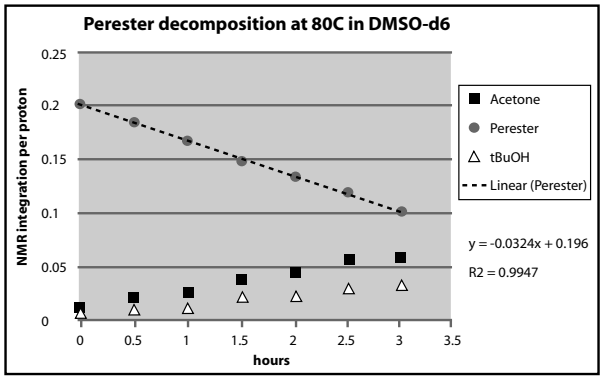

Figure 1. NMR characterization of thermal reactivity of perester group at 80°C in DMSO-d6. Decane is added as a standard.

HEALING OF VASCULAR DEVICES

Tuesday, October 25, 2005

11:15 - 11:45 a.m.

Invited Speaker

HEALING OF VASCULAR DEVICES

***Nicolas Chronos*, American Cardiovascular Research Institute**

Many theories or mechanisms of adhesion have been proposed to explain

BIOGRAPHY OF INVITED SPEAKER

NICOLAS CHRONOS

American Cardiovascular Research Institute
404-843-6067
nchronos@acrionline.org

Nicolas Chronos, M.D. is the Chief Medical and Scientific Officer of the American Cardiovascular Research Institute (ACRI) and the Saint Joseph's Research Institute.

In June of 1999, the Atlanta Cardiology Group, P.C. and Cardiology of Georgia, P.C. jointly founded ACRI, a non-profit organization, to promote and improve the comprehensive care of the cardiovascular patient. ACRI was created to integrate preclinical and clinical research programs to enhance understanding of the pathophysiology of cardiovascular diseases. Dr. Chronos currently oversees approximately 60 preclinical trials at ACRI and 30 clinical trials at the Saint Joseph's Research Institute.

Dr. Chronos received a Bachelors degree in Medicine and Surgery from the Royal Free Hospital School of Medicine in London in 1987. Following, he trained in cardiology and subsequently interventional cardiology under the direction of Professors Ulrich Sigwart and Anthony Rickards at the Royal Brompton National Heart and Lung Institute.

In 1992, he was awarded a British Heart Foundation International Fellowship and moved to the United States to continue his research in thrombosis and interventional cardiology at Emory University School of Medicine under the direction of Spencer B. King, III, M.D. He was named Director of Research at the Andreas Gruentzig Cardiovascular Center at Emory University Hospital in 1997.
Dr. Chronos is a Fellow of the Royal College of Physicians (London), Fellow of the American College of Cardiology, and Fellow of the European Society of Cardiology, and Fellow of the American Heart Association. He has served on the Data Safety Monitoring Board (ClinTrials Research), NIRVANA (Boston Scientific), and SCORES (Scimed Life Systems).

He was named Co-National Principal Investigator with Professor Mike Simmons on the FIRST study, the largest human clinical trial of angiogenesis. Currently Dr. Chronos is conducting two large clinical trials that use autologous or umbilical cord stem cells to repair damaged cardiac muscle. Dr. Chronos has also served on the Steering Committees for various studies RFPMR (Boston Scientific), TARGET (Merck & Co., Inc.), and FIRST (Chiron Corporation). Dr. Chronos has published over 200 peer-reviewed papers. His publications include articles in the Journal of the American College of Cardiology, Circulation, Heart and European Journal of Cardiology. He is an editorial consultant to the New England Journal of Medicine, British Heart Journal, Clinical Science, the Journal of the American College of Cardiology, Circulation, Heart, Atherosclerosis, Catheterization and Cardiovascular Interventions, Stent, Thrombosis Research, Cardiovascular Radiation Medicine, Journal of Stem Cells and other distinguished peer reviewed journals.

PERIPHERAL VASCULAR DISEASE TREATMENTS

Tuesday, October 25, 2005

1:30 - 3:00 p.m.

1:30 - 2:00 p.m.	**INVITED SPEAKER** **Novel Protein Therapies for Vascular Tissue Engineering** ***Eric Brey, Illinois Institute of Technology***
2:00 - 2:20 p.m.	Heparinized ePTFE Vascular Grafts: Development, Characterization, and Clinical Application for Infrainguinal Arterial Bypass *John Fisher, W.L. Gore & Associates, Inc.*
2:20 - 2:40 p.m.	Membrane-Mimetic Films Containing Thrombomodulin and Heparin Inhibit Tissue Factor-Induced Thrombin Generation in a Flow Model *Elliot Chaikof, Emory University*
2:40 - 3:00 p.m.	Fabrication of Hollow Alginate Fibers for Cellular Encapsulation *Philip Brown, Clemson University*

Invited Speaker

NOVEL PROTEIN THERAPIES FOR VASCULAR TISSUE ENGINEERING

***Eric Brey**, Ph.D., Illinois Institute of Technology*

The science of tissue engineering has provided hope for producing viable, patient-specific tissue substitutes. However, it has been difficult to translate this promise into clinical success. The ability to control endothelial proliferation and blood vessel formation would be of great benefit to tissue engineering therapies, with applications in promoting endothelialization of grafts and denuded vessels and stimulating extensive microvascular network formation in ischemic tissues or scaffolds. Success in these applications will most likely require strategies tailored to the specific tissue microenvironments. Using protein technologies we have developed 1) new proteins designed to localize in specific tissue environments and 2) methods for assembling natural 3D hydrogels with components specific to the targeted tissue. In combination with novel quantitative *in vitro* and *in vivo* assays we are characterizing the ability of these proteins to stimulate rapid and sustained neovascularization.

Using site-directed mutagenesis, Howard P. Greisler's lab at Loyola University Medical Center has shown that mutations in fibroblast growth factor-1 (FGF-1) can increase resistance to degradation (R136K mutant) and enhanced mitogenicity (FGF-1^{cys-} mutant). In collaboration with his lab, we have shown that FGF-1^{cys-} is a more potent angiogen *in vitro* and *in vivo*. We have combined these mutants with targeting approaches by synthesizing chimeric mutants with peptide sequences designed to localize to specific tissue microenvironments. Phage display libraries have been used to show that the asparagine-glycine-arginine (NGR) motif binds a specific CD13 isoform expressed on endothelial cells (ECs) in response to angiogenic stimuli. We have shown that this isoform is expressed by ECs in diabetic ischemic tissues. We synthesized wild type (NGR-FGF-1) and mutant (NGR-FGF-1^{cys-}) chimeras. These NGR chimeras retain the mitogenic properties of the base protein but bind to CD13 expressed by ECs in an isoform dependent manner. In addition, we have designed and synthesized an FGF-1 mutant chimera designed to bind collagen (collagen binding domain (CBD)-R136K) and promote endothelialization in areas of exposed collagen such as following angioplasty or vein bypass grafting. This mutant chimera specifically binds collagen and stimulates EC migration through collagen gels significanlt greater than FGF-1 or R136K.

This work illustrates how rational approaches allow the design of proteins with unique biological properties and localization within specific tissue microenvironments.

BIOGRAPHY OF INVITED SPEAKER

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Angiogenesis, tissue engineering, endothelial cell-tumor interactions, microvascular imaging

Selected Publications:

Brey, E.M., McIntire L.V., Johnston, C.M., Patrick, Jr., C.W. "Three-Dimensional, Quantitative Analysis of Desmin and Smooth Muscle Alpha Actin Expression During Angiogenesis." Annals of Biomedical Engineering. Accepted 2004.

Lalani Z., Wong M., Brey E.M., Mikos A.G., Duke P.J., Johnston C. "Spatial and Temporal Localization of Secretory IgA in Healing Tooth Extraction Sockets in a Rabbit Model." Journal of Oral and Maxillofacial Surgery. In Press.

Fisher J.P., Lalani Z., Bossano C.M., Brey E.M., Demian N., Johnston C.M., Dean D., Jansen J.A., Wong M.E.K., Mikos A.G., "Effect of Biomaterial Properties on Bone Healing in a Rabbit Tooth Extraction Socket Model." Journal of Biomedical Materials Research. 68A:428-438, 2004.

Kloc M., Bilinski S., Dougherty M.T., Brey E.M., Etkin L.D. "Formation Architecture and Polarity of Female Grmline Cyst in Xenopus." Developmental Biology. 266:43<ETH> 61, 2004.

Lalani Z., Wong M., Brey E.M., Mikos A., Duke P.J. Spatial and Temporal Localization of TGF-b1, BMP-2, and PDGF-A in Healing Tooth Extraction Sockets in a Rabbit Model. Journal of Oral and Maxillofacial Surgery 61:1061-1072, 2003.

Brey E.M., Lalani Z., Johnston C., Wong M., McIntire L.V., Duke P.J., Patrick Jr. C.W. Automated Selection of DAB-Labeled Tissue for Immunohistochemical Quantification. The Journal of Histochemistry and Cytochemistry 51(5):575-584, 2003.

Brey E.M., King T.W., Johnston C., McIntire L.V., Reece G.R., Patrick Jr. C.W. A Technique for Quantitative Three-Dimensional Analysis of Microvascular Structure. Microvascular Research 63(3):279-294, 2002.

King T.W.*, Brey E.M.*, Youssef A., Johnston C., Patrick Jr. C.W. Quantification of Vascular Density Using a Semiautomated Technique for Immunostained Specimens. Analytical and Quantitative Cytology and Histology (* equal contribution as first author). 24:39-48, 2002.

Kloc M., Dougherty M.T., Bilinski S., Chan A.P., Brey E., King M.L., Patrick Jr. C.W., Etkin L.D. Three Dimensional Ultrastructural Analysis of RNA Distribution within Germinal Granules in Xenopus. Developmental Biology 241: 79-93, 2002.

Brey E.M., McIntire L.V., King T.W., Reece G.P., Johnston C., Patrick Jr. C.W. Quantitative 3D Analysis Of Microvascular Networks In Tissue Engineering. The Internet Journal of Plastic Surgery 2001 Vol1N1 <http://www.ispub.com/journals/IJPS/Vol1N1/3d.html> .

Brey E.M., Patrick Jr. C.W. Tissue Engineering Applied to Reconstructive Surgery. IEEE Engineering in Medicine and Biology October/September: 122-124, 2000. Cheng M.H., Chen H.C., Wei F.C., Su S.W., Lian S.H., Brey E. Devices for Ischemic Preconditioning of the Pedicled Groin Flap. Journal of Trauma 48: 552-557, 2000.

Brey E.M., Ralston P.A.S. Comparison of Dead Time Compensation Schemes for Discrete Implementation of the Q-PID Algorithm. ISA Transactions 38: 171-183, 1999.

HEPARINIZED ePTFE VASCULAR GRAFTS: DEVELOPMENT, CHARACTERIZATION, AND CLINICAL APPLICATION FOR INFRAINGUINAL ARTERIAL BYPASS

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Autologous saphenous vein (ASV) is considered the best choice for peripheral vascular reconstructions; however, many patients in need of such procedures do not have adequate ASV. Although expanded polytetrafluoroethylene (ePTFE) vascular grafts have been used successfully as peripheral arterial bypasses for nearly three decades, early failure, due in part to thrombus formation on the luminal surface, remains problematic particularly in distal applications with impaired run-off. Thus, significantly enhancing clinical performance of small-diameter vascular grafts has remained a primary objective of applied clinical research. Our approach has been to improve thromboresistance of ePTFE vascular grafts via covalent endpoint immobilization of bioactive heparin on the luminal surface microstructure.

The heparin surface treatment incorporates a basement-layer coating of the luminal surface ePTFE microstructure and subsequent covalent endpoint immobilization of derivatized heparin using Carmeda® BioActive Surface (CBAS) technology. The molecular modification yields uniformly distributed, stably bound immobilized heparin on only the luminal aspect while the nanometer-scale permits conservation of the ePTFE microarchitecture without affecting mechanical integrity.

Chemical and physiological stability routinely are of interest when considering heparinized vascular grafts for peripheral bypass. Heparin immobilized on ePTFE grafts was stable in studies during which < 20 IU eluted after 7-day continuous-flow in vitro perfusion and pre- and post-elution heparin activities were not significantly different ($p < 0.05$). Long-term heparin bioactivity in vivo was demonstrated on heparinized ePTFE grafts explanted from canines at 1, 2, 4, 8, and 12 weeks. Activity levels on explanted heparinized grafts at each time point ranged from 15-25 pmol AT/cm2 and did not differ significantly ($p > 0.05$).

Results of preliminary in vivo studies clearly indicated improved thromboresistance and improved patency resulting from endpoint heparin immobilization. In acute canine carotid artery interposition experiments, heparinized ePTFE grafts remained patent and had significantly greater thrombus-free luminal surface area ($p < 0.05$) compared to nonheparinized ePTFE controls. In chronic canine femoral artery interposition experiments, 6-month patency of heparinized ePTFE grafts was significantly greater ($p < 0.05$) than nonheparinized ePTFE controls.

Clinically, over 8,000 heparinized GORE-TEX® ePTFE vascular grafts, marketed under the brand name PROPATEN, have been implanted for treatment of complications of peripheral vascular disease. To date, clinical data indicate markedly improved patency and limb salvage for infrainguinal bypasses performed with heparinized ePTFE compared to clinical performance of nonheparinized prosthetic grafts (e.g. above-knee patency ≈ 77-82%) over the past several decades. Recently, some authors have reported 1-year above-knee and below-knee primary patencies of 91% and 92% for heparinized ePTFE bypasses and limb salvage rates of 95-98%. These data suggest that the localized thromboresistance imparted to ePTFE vascular graft surfaces by CBAS endpoint immobilization of heparin represents a significant advancement in the state of the art of prosthetic arterial bypass in clinical treatment of peripheral vascular disease.

Key Words: ePTFE, Heparin, Thromboresistance

MEMBRANE-MEMETIC FILMS CONTAINING THROMBOMODULIN AND HEPARIN INHIBIT TISSUE FACTOR-INDUCED THROMBIN GENERATION IN A FLOW MODEL

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Membrane-mimetic thin films containing thrombomodulin (TM) and/or heparin were produced and their capacity to inhibit thrombin generation evaluated in a continuous flow system. Tissue factor along with thrombomodulin and heparin were immobilized in spatially restricted zones as components of a membrane-mimetic film. Specifically, TF was positioned as an upstream trigger for thrombin generation and TM and/or heparin positioned over the remaining downstream portion of test films. Peak and steady state levels of thrombin were decreased by antithrombin III (ATIII), as well as by surface bound heparin and TM. Although physiologic concentrations of ATIII have the capacity to significantly inhibit thrombin activity, surface bound TM and heparin nearly abolished steady state thrombin responses. In particular, surface bound TM appears to be superior to heparin in reducing local thrombin concentrations. These studies are the first to demonstrate the additive effect of surface bound heparin and TM as a combined interactive strategy to limit TF-induced thrombin formation.

FABRICATION OF HOLLOW ALGINATE FIBERS FOR CELLULAR ENCAPSULATION

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Implant devices are prone to three major complications, one of which is incomplete/ non-specific cellular healing leading to the ultimate failure of the implantation. It is the objective of this ongoing project to develop novel porous alginate fiber membranes (and films) containing endothelial cell that can deliver appropriate chemicals to the body, thereby improving the overall healing characteristics of the implantable materials. The developed novel biomaterial will emulate some of the normal healing processes of native tissue by direct incorporation of specific cells (i.e., endothelial cells or EC). Alginate was selected because of its non-toxicity, non-carcinogenic non-allergenic, porous, high liquid absorbance and haemostatic effect on wounds. Hollow fiber spinning was conducted to make hollow and porous alginate membrane fibers via a dry-jet wet spinning system. Hollow fibers were obtained by extruding a degassed sodium alginate solution at the desired rate through a tube-in-orifice type spinneret into chosen coagulants. The internal coagulant (required to produce the hollow fiber structure) was injected through the side capillary of the tube-in-orifice spinneret and controlled by a syringe pump to give the fiber its characteristic hollow shape and to control (to some extent) the final fiber morphology. During the dry jet wet spun process the nascent fiber was passed through an air gap before entering the coagulation bath. The fibers were then taken from the coagulation bath to wind up roller. Wet spinning and dry jet wet spinning of alginate hollow fibers involves a complicated combination of spinning variables including polymer solution extrusion rate, internal coagulant injection rate, air gap (if used), windup speed, residence time in the coagulation bath, temperature of the polymer solution, temperature of the coagulants and the composition of the coagulation baths. The different approaches explored to obtain fibers of different structures and morphology will be discussed. Morphological characterization of the produced fibers has been made using SEM. While hollow fibers have certain practical advantages in terms of cellular incorporation "pseudo bicomponent" sheath core alginate fibers with different inner and outer morphology have also been spun and characterized. This work is an interdisciplinary, multi-center effort. The future development of cellular encapsulation methodology and EC cell seeding methods are currently being explored.

Key words
Alginate hollow fibers, dry jet wet spinning, cell incorporation

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MATERIAL CHARACTERIZATION

Wednesday, October 26, 2005

9:00 - 10:30 a.m.

9:00 - 9:30 a.m.	**INVITED SPEAKER** **Microscopy of Soft, Conductive, and Bioactive Materials for Interfacing Electronic Devices with Living Tissue** ***David C. Martin, University of Michigan***
9:30 - 9:50 a.m.	Morphology of Polymer Blend Coatings for Drug Delivery *Klaus Wormuth, SurModics, Inc.*
9:50 - 10:10 a.m.	Evaluation of Acrylate-Based Block Copolymers Prepared By Atom Transfer Radical Polymerization as Matrices for Paclitaxel Delivery From Coronary Stents *Robert Richard, Boston Scientific Corporation*
10:10-10:30 a.m.	Human Platelet Responses to Silicon-Alloyed Pyrolytic Carbons *Steve Goodman, 10H, Inc.*

Invited Speaker

MICROSCOPY OF SOFT, CONDUCTIVE AND BIOACTIVE MATERIALS FOR INTERFACING ELECTRONIC DEVICES WITH LIVING TISSUE

David C. Martin, Professor of Materials Science and Engineering, Biomedical Engineering, and Macromolecular Science and Engineering, The University of Michigan Ann Arbor, MI

Our research group has been interested in polymer coatings designed to improve the long-term reliability and biocompatibility of electronic devices with living tissue. Of particular interest to us have been microfabricated neural prosthetic devices intended for recording and stimulation of the Central Nervous System (CNS). Our recent efforts have focused on conducting polymers that can be electrochemically deposited on the surfaces of probe electrodes intended for implantation into the cortex. These materials can be tailored to have precisely controlled microstructures by using various templates and biologically-active counterions during deposition. The mechanical properties of the polymer coatings are much more compliant than the rigid biosensor substrate, improving interactions with the soft tissue. The coatings also facilitate charge transport, resulting in significantly lower impedances. We have examined the microstructure of these materials using a variety of microscopic analysis techniques including optical microscopy (OM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), low dose high-resolution electron microscopy (HREM), atomic force microscopy (AFM) and nanoindentation. We have also used a low voltage electron microscope (LVEM). The LVEM operates at 5 kV and provides high contrast images of polymer and organic molecular thin films. It has a compact size and is capable of operating in SEM, TEM, and STEM modes.

BIOGRAPHY OF INVITED SPEAKER

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Professor Martin received a B. S. in Materials and Metallurgical Engineering Summa Cum Laude in 1983, and a M. S. in Macromolecular Science and Engineering in 1985 from the University of Michigan. He then received a Ph.D. in 1989 from the University of Massachusetts at Amherst, under the direction of Prof. Edwin L. Thomas, now at MIT. He was a Research Scientist at DuPont Central Research till 1990, when he moved to a position in the Materials Science and Engineering Department at the University of Michigan. Prof. Martin was named a NSF National Young Investigator in 1994, and spent a year as a Humbodt fellow in the laboratory of Prof. Gehard Wegner in the Max Planck Institute for Polymer Research in Mainz, Germany. Martin was named a fellow of the American Institute of Medical and Biological Engineering in 2005. He research interests include high resolution electron microscopy of ordered polymer and organic molecular thin films, surface deformation of polymers, and the design and characterization of conducting polymers for interfacing electronic devices with living tissue, with a particular emphasis on microfabricated neural prosthetics.

MORPHOLOGY OF POLYMER BLEND COATINGS FOR DRUG DELIVERY

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Introduction

Combination of the physical action of medical devices with the chemical action of a drug eluting coating has, in the example of drug eluting stents, significantly advanced the treatment of cardiovascular disease. Critical to successful application of drug eluting coatings is tailoring the polymer matrix to incorporate high concentrations of drug, to control the elution of the drug, and to withstand the deformation of the device upon insertion into the body. Polymer blend coatings for drug delivery often offer advantages not found in single polymer coatings: ability to tune drug elution rates and mechanical properties by varying the ratio of the two polymers. However, the complexity introduced by increasing the number of components in the coating makes a thorough understanding of the morphology of the blend more difficult to unravel compared to single polymer coatings. Here we apply light, Raman, infrared, scanning electron and atomic force microscopies to decipher the morphology of polymer blend coating incorporating high concentrations of drug.

Experimental

Films of dexamethasone in blends of poly(alkyl methacrylate) polymers were cast on glass slides or spray coated onto stents. Dark field light microscopy helps examine the cloudiness of the coatings, and polarized light microscopy aides in the determination of crystalline drug. Scanning confocal Raman microscopy generates Raman spectra at each pixel in a cross-sectional or planar image, which when deconvoluted using reference spectra generates images of the distribution of individual components within the coating. In this way, the degree of mixing of the drug and polymers, any layering of materials, in addition to any changes in drug polymorphs was measured. Infrared microscopy in transmission-reflection-transmission mode is one way to determine the uniformity of the overall coating composition over larger areas. Light, Raman, and infrared microscopy probe into and throughout the coating thickness, whereas high resolution scanning electron and atomic force microscopy probe only the surface properties of the coatings. Atomic force microscopy yields the distribution of drug and polymer directly at the coating surface, as measured by changes in the interaction (energy dissipation) of the AFM tip with the surface. High resolution low-voltage scanning electron microscopy reveals the smoothness of the surface, and the morphology of any crystals of drug on the coating surface.

Results and Discussion

Films of dexamethasone in blends of poly(butyl methacrylate) and poly(lauryl methacrylate) (43.5/43.5/13 by weight) cast onto glass slides show regions of crystallinity under polarized light, in addition to regions with small lens-shaped objects. Raman analysis finds the crystals to be composed of pure dexamethasone, and the lens-shaped objects to be regions rich in poly(lauryl methacrylate) dispersed in a sea of poly(butyl methacrylate). Amorphous dexamethsone strongly partitions into poly(butyl methacrylate), with little found in poly(lauryl methacrylate) regions.

Individual spray droplets deposited onto glass slides show fine mixing of drug and polymers. Atomic force microscopy shows small (~100 nm) blobs of poly(lauryl methacrylate) dispersed in poly(butyl methacrylate), with coarser mixing towards the edges of the droplets. Initially, no crystalline dexamethasone is found, but with time, small crystals of drug grow at the edges of the dried droplets.

Spray coatings on stents also exhibit fine surface textures under examination with the scanning electron microscope, the textures likely generated as spray droplets impact, flow and dry. In a few areas, small crystals of drug were found at the surface of the coating. The local thickness and stratification of the drug/polymer blend coating overcoated with a layer of poly(butyl methacrylate) alone is determined with Raman microscopy, and more global homogeneity of this overcoated layer found to be non-uniform as determined by measuring the drug/polymer ratio using infrared microscopy.

Conclusions

Mixtures of dexamethasone in blends of poly(alkyl methacrylates) show strikingly different morphology depending upon how the coating is produced: coarse mixing in cast films, and fine mixing in sprayed films. Strong preferential partitioning of dexamethasone into poly(butyl methacrylate) compared to poly(lauryl methacrylate) is found. Spray coatings show fine mixing, even with a high concentration of dexamethasone within the coating. The evidence here suggests these spray coatings are supersaturated in drug. In drug elution testing, the initial burst of the drug from spray coatings correlates strongly with the homogeneity of mixing of the drug within the polymer blend matrix.

EVALUATION OF ACRYLATE-BASED BLOCK COPOLYMERS PREPARED BY ATOM TRANSFER RADICAL POLYMERIZATION AS MATRICES FOR PACLITAXEL DELIVERY FROM CORONARY STENTS

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Introduction

Device-based drug delivery is highly dependent on polymeric materials to serve as coatings and matrices to release the therapeutic in a controlled fashion. The drug coated coronary artery stent is an excellent example of a device-based drug delivery product which relies on a polymer to serve as the drug depot and release it in a controlled fashion after the implantation is completed.[1-2] The clinical success of drug eluting coronary stents has been well documented in the recent literature.[3-5] The mechanical requirements of a drug delivery coating for this device include elasticity and toughness to preserve the integrity of the coating. The use of block copolymers for this purpose has been reported previously and is a proven approach to realize the key mechanical properties required.[6] In addition to good mechanical stability, polymer coatings for drug delivery applications need to be compatible with the in vivo environment to which they will be exposed. This requirement is especially critical for the coronary stent application, where the polymer coating is exposed to both vascular tissues of the artery as well as flowing blood. Along with biocompatibility, the polymer must also be biostable and not breakdown, especially during the time when drug is being delivered. Biodegradation could potentially affect the release rate or introduce inflammatory degradants during the lifetime of the implant.[7]

Atom transfer radical polymerization (ATRP) is a versatile technology for preparing block copolymers with well controlled chemical composition and morphology and is applicable to monomers with a wide range of polarity.[9-12] This technology has been shown to also allow for the production of a vast range of copolymer morphologies with phase separation at the nanoscale. Such morphologies are known to result in materials with unique mechanical properties, providing additional modes for regulating the release of and for producing surfaces that are compatible with the in vivo environment.

The objective of this study was to evaluate a series of elastomeric acrylate-based block copolymers synthesized by ATRP to determine their ability to release paclitaxel (PTX) after coating onto coronary stents. These copolymers were composed of immiscible blocks that resulted in unique nano-phase separated materials. In addition, the release rates were evaluated both before and after exposure to doses of electron beam (e-beam) radiation representative of that typically used to sterilize medical devices. The molecular weight distribution of the copolymers following e-beam exposure were measured to determine their stability to ionizing radiation.

Experimental

Chemicals, copolymer syntheses and characterizations have been described elsewhere.[13-16] Paclitaxel release testing, atomic force microscopy, stent expansion and scanning electron microscopy (SEM) measurements as well as coronary stent coating methods have been previously published.[1,2]

Results and Discussion

The multi-block copolymers shown in Table 1 were successfully prepared using previously published ATRP methods.[13-16] ATRP is a convenient method to produce multiblock copolymers which have immiscible hard and soft segments leading to elastomeric properties that are ideal for coatings applied to expandable devices such as coronary stents.

After coating coronary stents with each formulation, the mechanical integrity of the acrylate-based coatings were evaluated via examination by SEM both before and after expansion. It was found that samples with a hard block content greater than 50% by weight typically produced stent coatings that resulted in integrity failure upon expansion of the stents. The drug release testing was performed on non-expanded stents to avoid drug release effects caused by coating failure.

Table 1. *Chemical Composition of Block Copolymers Coated onto Coronary Stents*

Polymer Composition	Block molecular weights g/mole	Mw/Mn
P(MMA-BA-MMA)	17.3k-48.9k-17.3k	1.07
P(MMA-BA-MMA)	29.3k-41.8k-29.3k	1.25
P(MMA-BA-MMA)	23k-28k-23k	1.4
P(MMA-LA-MMA)	15k-26.5k-15k	1.22
P(IBA-BA-IBA)	21.8k-41.8k-21.8k	1.46
P(IBA-BA-IBA)	17.5k-55k-17.5k	1.08
P(IBA-BA-IBA)	10.9k-55k-10.9k	1.08
P(IBA-LA-IBA)	11.9k-26.5k-11.9k	1.19
P(STY-LA-STY)	14.6k-24.4k-14.6k	1.26
P(SAN-BA-SAN)	19.8k-49k-19.8k	1.40
P(STY-BA-STY)	19.8k-55k-19.8k	1.10
P(STY-BA-STY)	10.8k-55k-10.8k	1.08
P(BA-S)3 tri-arm star	$(45k\text{-}6k)_3$	1.13
P(BA-S)	28k-12k	1.15

MMA = methyl methacrylate; BA = butyl acrylate; LA = lauryl acrylate; IBA = isobornyl acrylate; STY = styrene; SAN = styrene-acrylonitrile

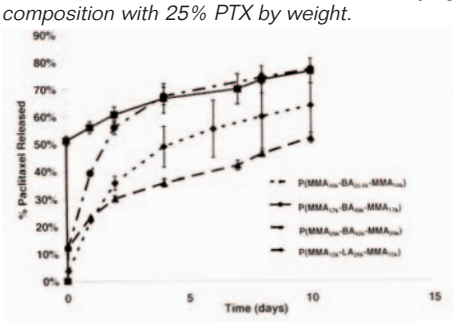
(continued)

The morphology of the copolymer coatings both with and without drug was evaluated by AFM. This was performed on spin-coated silicon wafers. The images were obtained from films consisting of copolymer alone or containing 10 and 25% PTX by weight. The images showed the expected phase-separated morphology, however it is not well defined due to the non-equilibrium conditions of these films resulting from rapid evaporation of the solvents when producing the coatings. These conditions are representative of the process used to coat coronary stents and would be expected to result in similar morphological structure. It was also observed that the triblocks containing either polystyrene or poly(isobornyl acrylate) showed evidence of phase separated PTX particles on the surface, whereas no PTX could be identified in the copolymer containing poly(methyl methacrylate) end blocks.

Differential scanning calorimetry (DSC) analysis was conducted to confirm the phase separation of the block copolymers by the presence of two distinct T_g's. In addition, DSC was conducted on samples of copolymer containing PTX. In all cases, it was determined that the PBA T_g increased as the PTX content was increased whereas the end blocks showed no significant change. The T_g of amorphous paclitaxel is reported to be 151 °C.[16] This indicates that the PTX is to some degree miscible with the PBA mid blocks.

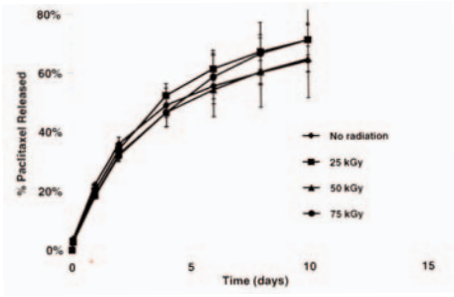
The PTX release profiles for formulations with PMMA hard blocks with PBA or PLA soft blocks evaluated with 25% PTX are shown in Figure 1. All compositions appear to give the same release rate after the 2 h data point. For these samples, the M_n ranged from approximately 46,000 to 100,000 g/mo,l and the weight percent MMA varied from 41 to 58. The data indicate that at 25% PTX loading, the release rate is independent of % MMA, the nature of the mid-block, and molecular weight. It was observed that one of the PMMA/PBA block copolymers released approximately 50% of the PTX within 2 h, which was substantially different than the other similar copolymers tested. Since the M_n and % MMA for this sample are bracketed by the other formulations, it is hard to attribute this behavior to composition or molecular weight.

Figure 1. *Paclitaxel release from triblock copolymers containing PBA or PLA soft blocks and MMA hard blocks of varying molecular weight and composition with 25% PTX by weight.*



To examine the effects of radiation on the release rate of PTX, copolymer/ drug coated stents were subjected to ionizing e-beam radiation and subsequently tested for PTX release. Figure 2 shows the PTX release rates for a PBA/PMMA copolymer containing 25% by weight PTX after exposure to 0, 25, 50, and 75 kGy doses of e-beam radiation. It was found that the PTX release rates were essentially independent of radiation dose. Even though the PMMA blocks contain quaternary carbons and therefore would be expected to experience some molecular weight reduction upon irradiation, the reduction does not appear to cause a measurable change in the PTX release rate. The stability of the PBA/PMMA material and the corresponding PTX release rate is attributed to the superior radiation stability of the PBA mid-block and may also be related to the small size of the less radiation stable PMMA end blocks.

Figure 2. *Effect of radiation dose on the KDR of PTX from P(MMA-BA-MMA) with 25% PTX by weight.*



Conclusions

The release of PTX from acrylic based block copolymers appears to be independent of the soft mid-block for PBA and PLA. In the case of PMMA end-blocks, no visible PTX was observed by AFM, indicating more miscibility between the components, although no miscibility between PMMA and PTX could be detected by DSC analysis. The PTX release from the acrylate-based block copolymers appears to be unchanged by radiation sterilization for the copolymers and doses evaluated in this study. The radiation stability of the mid-block in triblock copolymers therefore is more important in preserving the PTX release profile.

References

(1) Ranade, S.; Miller, K.; Richard, R.; Chan, K.; Allen, M.; Helmus, M. J. Biomedical Mats. Res. 2004, 71A, 625.
(2) Richard, R.; Barry, J.; Kamath, K.; Miller, K.; Ranade, S.; Schwarz, M., 31st Annual Meeting of the Controlled Release Society, Honolulu, Hawaii, USA, 2004 June 12-16.
(3) Stone, G. W.; Ellis, S. G.; Cox, D. A.; Hermiller, J.; O'Shaughnessy, C.; Mann, J. T.; Turco, M.; Caputo, R.; Bergin, P.; Greenberg, J.; Popma, J. J.; Russell, M. E.; N. Engl. J. Med. 2004, 350, 221-231.
(4) Stone, G. W.; Ellis, S. G.; Cox, D. A.; Hermiller, J.; O'Shaughnessy, C.; Mann, J. T.; Turco, M.; Caputo, R.; Bergin, P.; Greenberg, J.; Popma, J. J.; Russell, M. E., Circulation 2004, 109, 1942-1947.
(5) Tanabe, K.; Serruys, P. W.; Grube, E.; Smits, P. C.; Selbach, G.; van der Giessen, W. J.; Staberock, M.; de Feyter, P.; Muller, R.; Regar, E.; Degertekin, M.; Ligthart, J. M. R.; Disco, C.; Backx, B.; Russell, M. E, Circulation 2003, 107, 559-564.
(6) Ranade, S. V.; Richard, R.; Helmus, M. Acta Biomaterialia, 2005, 1, 137.
(7) Steckel, M.; Richard, R.; Strauman, P.; Barry, J. Society for Biomaterials Philadelphia PA, October 2004.
(8) Schwarz, M.; Ranade, S., 31st Annual Meeting of the Controlled Release Society, Honolulu, Hawaii, USA, 2004 June 12-16
(9) Wang, J.-S.; Matyjaszewski, K. J. Am. Chem. Soc. 1995, 117, 5614-5615.
(10) Matyjaszewski, K.; Xia, J. Chem. Rev. 2001, 101, 2921-2990.
(11) Kamigaito, M.; Ando, T.; Sawamoto, M. Chem. Rev. 2001, 101, 3689-3745
(12) Beers, K. L.; Woodworth, B.; Matyjaszewski, K. J. Chem. Educ. 2001, 78, 544-547.
(13) Beers, K. L.; Matyjaszewski, K. J. Macromol. Sci., Pure Appl. Chem. 2001, A38, 731-739.
(14) Shipp, D. A.; Wang, J.-L.; Matyjaszewski, K. Macromolecules 1998, 31, 8005-8008.
(15) Tsarevsky, N. V.; Sarbu, T.; Goebelt, B.; Matyjaszewski, K. Macromolecules 2002, 35, 6142-6148.
(16) Liggins, R.T.; Hunter, W.L.; Burt, H. M. J. Pharm. Sci. 1997, 86, 1458-1463.

HUMAN PLATELET RESPONSES TO SILICON-ALLOYED PYROLYTIC CARBONS

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Introduction

Pyrolytic carbon (PYC) containing about 7 weight-percent silicon is widely used in mechanical heart valves, where it has demonstrated a high level of blood compatibility (1). The Si, present as SiC, is included since it is believed to enhance wear resistance and thus durability. It has been suggested (2), based upon early Gott ring tests (3) that SiC reduces the blood compatibility of PYC. If so, then reducing the Si concentration would be expected to provide improved blood compatibility (2). In the following study, we have evaluated this by preparing PYC materials with three different level of Si, and examining the in vitro response of human platelets to these materials using a well-established static in vitro platelet assay (4-6).

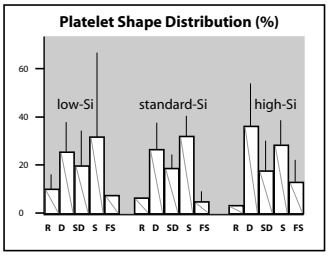
Materials and Methods

Clinical-grade PYC leaflets were formed using fluidized-bed chemical vapor deposition of propane at St. Jude Medical. Methyltrichlorosilane (MTCS) vaporized by helium was the source of the silicon. Low and high Si PYC leaflets were fabricated by shutting off or increasing the MTCS flow, respectively. Leaflets were machined and polished, cut into 10 x 10 mm specimens, and then cleaned and steam sterilized using clinical valve methods. Elemental composition was analyzed by EDX (Oxford ISIS on a Leica S-430I) showing that the standard clinical Si-alloyed PYC contained 7.5 wt. % Si, low Si PYC was <0.5% Si, and the high Si PYC was 14.2% wt. % Si.

The adhesion of column-washed human platelets obtained from healthy volunteers was evaluated on the three PYC materials. Platelets in the presence of 1 mg/ml albumin were applied to pre-hydrated surfaces at 37°C for 10 minutes and 45 minutes to examine early and later stage platelet responses. The preparation was then gently rinsed with buffer to remove non-adherent cells, fixed with glutaraldehyde and osmium, and prepared for SEM using the critical point procedure. Random micrographs were obtained at 1,000-3000X using a JEOL JSM 6320 FEV low voltage field emission SEM. Micrographs were analyzed for multiple platelet parameters including (projected) spread areas, surface area coverage, number per unit area, quantitative shape analysis, and measures of surface aggregation including cohesion-adhesion ratios and the number of thrombi-like micro-aggregates (6).

Results

Platelet responses to the three PYC materials were comparable to previous reports: Adherent platelets were extremely well spread, closely followed submicron PYC contours, and formed extremely few aggregates or microthrombi-like structures. This is in dramatic contrast to platelet responses to control polymers including NIH reference polyethylene and silicone rubber, and formvar (4-6). No significant differences with respect to Si concentration were observed for the number of platelets per unit area, cohesion/adhesion ratios, percent of the surface covered by platelets, and the numbers of micro-aggregates. Similarly, distributions of platelet shapes (mean + sd), from round (R) to fully spread (FS), at 45 minutes (figure) were also not significantly different.



Discussion

Varying the Si concentration over a range of <0.5 to 14 wt. % had no statistically detectable effect on the in vitro behavior of human platelets. While some slight differences were observed with some parameters, all effects were small and often variable in direction with respect to Si concentration. These results appear to agree historical Gott ring test work, where low-Si (unalloyed) and standard Si-PYC were not demonstrably different (although Si levels were not provided in the Gott report), while pure SiC coatings (very high Si levels), were highly thrombogenic (3). Therefore, the present results do not suggest that lowering (or raising) the Si concentration in PYC over reasonable ranges will improve the in vivo blood compatibility of this important clinical material.

References

1. KT Tweden, Cells and Materials 6, 319-337 (1996).
2. RB More, A Haubold, Cells and Materials 6, 273-279 (1996).
3. LD LaGrange, VL Gott, JC Bokros, MD Ramos, in Artificial Heart Program Conference Proceedings, FW Hastings, LT Harmison, RJ Hegyeli (eds.) Washington, D.C., (1969) pp. 47-58.
4. SL Goodman, KS Tweden, RM Albrecht, Cells and Materials 5, 15-30 (1995).
5. SL Goodman, KS Tweden, RM Albrecht, J. Biomed. Mater. Res. 32, 249-258 (1996).
6. SL Goodman, J. Biomed. Mater. Res. 45, 240-250 (1999).

HUMAN CELLULAR AND TISSUE-BASED THERAPIES

Wednesday, October 26, 2005

2:00 - 3:30 p.m.

2:00 - 2:30 p.m.	**INVITED SPEAKER** **Adult Derived Mesenchymal Stem Cells and Potential Clinical Applications** ***Russel Reiss, VA Hospital, University of Utah School of Medicine***
2:30 - 2:50 p.m.	Comparison of Bone Marrow and Blood Mononuclear Cell-Seeded Biological Matrices for Myocardial Tissue Repair *Jeremy Ollerenshaw, American Cardiovascular Research Institute*
2:50 - 3:10 p.m.	Engineering a Biohospitable Acellular Cardiovascular Tissue *Alyce Linthurst Jones, LifeNet*
3:10 - 3:30 p.m.	The Use of Adult Derived Adipose Stem Cells for Bone Regeneration *Kelly Kirker, Bacterin International, Inc.*

Invited Speaker

ADULT DERIVED MESENCHYMAL STEM CELLS AND POTENTIAL CLINICAL APPLICATIONS

G. Russel Reiss, M.D., Division of Cardiothoracic Surgery, VA Salt Lake City Health Care System, University of Utah School of Medicine

There are a variety of tissue and bone marrow-derived stem cells that exist in the adult. In addition, resident stem cells found throughout the body are being discovered and isolated everyday from various organs. Because of their "ease of use" in the laboratory, these adult derived stem cells, in particular, mesenchymal stem cells (MSCs), have become the stem cell of choice for tissue engineering applications.

For a biologic product such as a cell to be "research applicable" in the field of tissue engineering, the product must be readily harvestable and isolatable, or available from a stock source such as a cultured cell line. Next, these cells must possess characteristics that are in line with the goals of end production for the combined biomaterial-cell product. Lastly, these cells will have to be able to withstand the rigorous validation protocols involved in the FDA regulatory process.

The plasticity of MSCs is their greatest strength for the world of tissue engineering. They are now reliably able to be driven down differentiation pathways to form bone, muscle, fat etc… Many of these pathways are highly reproducible and easily followed in the lab with commercially available kits. With the wide spread application of biomatrices and biocompatible scaffolds such as hydrogels, MSCs have been combined with nearly ever major biologic delivery system to date for hundreds of potential clinical applications.

To be "clinically applicable" any biologic or cell product has to be produced under a good manufacturing practice (GMP) environment and tested by investigators certified in Good Clinical Practices and Human Subject Protection. This last hurdle can certainly be the biggest and should be the genesis for strategic alliances between the research, academic, and biotechnology sectors.

BIOGRAPHY OF INVITED SPEAKER

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G. Russell Reiss, M.D. is board certified cardiothoracic surgeon affiliated with the University of Utah and the George E. Wahlen Department of Veterans Affairs Medical Center. He directs a nationally funded stem cell research program performing both clinical trials and basic science research in the cellular treatment of ischemic heart disease. His background includes immunobiology and gene therapy. He has authored several scientific manuscripts and abstracts and presented as an invited speaker on such topics as the hurdles involved with the clinical application of stem cells, regenerative medicine and the bioentrepreneur, and working through the FDA. Dr. Reiss is a Sponsor-Investigator with the FDA currently with an active Investigational New Drug approval (IND) for use of adult stem cells for the treatment of coronary artery disease. He received his B.A. in Biology from La Salle University, his M.D. from Hahnemann University and completed a postdoctoral research fellowship in the Center for Gene Therapy under the mentorship of Darwin J. Prockop, MD, PhD. He is currently on the scientific advisory board of Bacterin International, Inc. and is a consultant to several other biotechnology companies and biologic divisions.

COMPARISON OF BONE MARROW AND BLOOD MONONUCLEAR CELL-SEEDED BIOLOGICAL MATRICES FOR MYOCARDIAL TISSUE REPAIR

***Jeremy Ollerenshaw*, Traci Goodchild, Benjamin Crumley**
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Introduction: Cell transplantation regimens are being used as therapeutic tools in myocardial tissue repair to increase muscle mass, limit wall thinning and prevent scar formation. However, a cell support scaffold may be required to aid localization and retention, and maintain cells viable during transplantation. To this end, appropriately seeded biological matrices have the potential to function as biocompatible tissue constructs. Additionally, as cardiac myocytes have no natural capacity for regeneration, sources of autologous progenitor cells such as bone marrow and peripheral blood cells are being evaluated as potential cell sources. To initiate development of seeded scaffold based therapies for myocardial repair, we have evaluated the growth potential of porcine blood mononuclear cells and bone marrow seeded onto allogeneic collagen-rich scaffolds *in vitro* for periods of up to 10 weeks, and monitored cellular colonization and matrix integrity.

Methods: Sixty ml aliquots of venous blood and two to five ml aliquots of bone marrow were drawn from adult pigs into EDTA collection tubes, and mononuclear cells were fractioned using Histopaque-1077 and centrifugation. Cells were subsequently washed in PBS and separately resuspended at a seeding density of approximately $2x10^6$ (blood) or $2x10^5$ (bone marrow) cells per 100ml of DMEM containing 10% FBS, antibiotics and approximately 3ml of 0.5mm^3 pieces of acellular porcine ureter in a 125ml capacity spinner flask culture system. Cultures were maintained for up to 10 weeks, and aliquots of tissue were removed periodically during media changes for histological processing and evaluation of H&E stained paraffin sections to determine cell growth and comparative morphology of peripheral blood cells and bone marrow cell populations.

Results: Preparation of the porcine ureter scaffold was designed to preserve the collagen structure and associated surface properties that may encourage cell conduction upon seeding. Within a 14-day seeding period, adhesion and growth of blood and bone marrow cells into the tissue matrix was evident. Cells were seen to cover the surface of tissue and also penetrate into the deeper layers to fully colonize the tissue scaffold. Over time, cells took on a spindle shaped morphology in alignment with the collagen structure of the scaffold substrate. Up to 10 weeks of culture, no discernible differences were seen in morphology between blood cells and bone marrow cells.

Conclusion: Acellular porcine ureter can support the adhesion, growth, and morphologic differentiation of both bone marrow and blood-derived cells, potentially through surface properties retained during this decellularization process. These data support further investigation into the utility of the more easily accessible circulating blood cells for preparation of *in vitro* cell seeding of acellular tissue scaffold intended for clinical cardiac repair. Transplantation of such constructs containing differentiated cells may initiate structural and functional regeneration of the diseased or damaged heart.

BIOGRAPHY OF SPEAKER

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Dr. Ollerenshaw is a Senior Staff Scientist with ACRI directing preclinical studies in cardiovascular device testing with a specific interest in the advancement of biological tissue-engineered scaffolds. He received his PhD in 1989 from the University of Leicester in the UK and has 20 years research experience in vascular and cardiovascular medicine including 10 years in the medical device industry. He has over 50 publications in the scientific literature, has received numerous grants for cardiovascular research and is an inventor of medical device patents the US and in Europe.

ENGINEERING A BIOHOSPITABLE ACELLULAR CARDIOVASCULAR TISSUE

***Alyce Linthurst Jones*, Colleen Roche, Allison O'Neal, Mark Moore, Lloyd Wolfinbarger, Jr.**
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Introduction: Numerous methods exist to render tissue acellular[1]. However, the resultant tissues are not always biohospitable to infiltrating cells and clinical failures were reported.[2] LifeNet has developed a patented process using N-lauroyl sarcosinate (NLS), Benzonase and ion exchange/adsorbant resins to engineer a biohospitable acellular cardiovascular tissue. To engineer a biohospitable cardiovascular tissue matrix, it is important to characterize the following: 1) the binding and release kinetics of processing reagents to/from the collagen matrix, 2) any biomechanical effects the process may have on the tissue, 3) the collagen matrix, and 4) the performance of the tissue *in vivo* under physiological conditions with respect to cellular infiltration, cell viability and stability of the extracellular matrix. These aspects were tested using NLS binding and release kinetics to/from cardiovascular tissue, burst testing, differential scanning calorimetry (DSC), and characterization of the acellular scaffold under physiological conditions in the 20-week female juvenile sheep model.

Methods: Pulmonary conduit tissue was rendered acellular using a patented decellularization technology called "devitalization," (U.S. Patent # 6,743,574). Tissue was placed in a chamber, and N-lauroyal sarcosinate (NLS), Benzonase and antibiotics were recirculated for 24-36 hours. Water was subsequently recirculated through both the tissue and also a chamber containing hydrophobic adsorbant resin and anion exchange resin for 24-36 hours. Finally, a proprietary preservation solution was recirculated for 24-36 hours. NLS binding and release kinetics were ascertained by circulating ^{3}H-NLS through the tissue to assess NLS uptake overtime and then rinsing the tissue and obtaining NLS release over time. Biomechanical effects were ascertained by following the ASTM D 3797-89 Standard Test Method for Bursting Strength of Knitted Goods, Constant-Rate-of-Transverse (CRT) Ball-Burst Test. The burst strengths of four cryopreserved tissues and four companion-devitalized tissues were compared. To characterize the integrity of the collagen at the molecular level, the onset temperatures and peak temperatures of fifteen cryopreserved and fifteen devitalized companion tissues were assessed using DSC. Finally, cryopreserved and devitalized tissues were implanted as patch grafts in the right ventricular outflow tract of juvenile sheep and then explanted after 20 weeks *in vivo*. The patches were assessed for dimensional changes by direct measurement under pressure. They were evaluated for recellularization by H&E and for calcification by Alizarin Red S. The patches were tested for apoptosis by TUNEL staining and for physiological activity by staining for Factor VIII and alpha smooth muscle actin. Finally, they were assessed by *in situ* hybridization for Type I collagen production.

Results: NLS binding and release kinetics studies revealed that equilibrium was attained after 24 hours. The maximum removal of NLS was achieved after rinsing for 24 hours, leaving 0.1% of the NLS behind. Ball burst testing revealed no statistically significant difference ($p < 0.05$) in burst strength between cryopreserved and devitalized conduits. The DSC assessment revealed that the onset and peak temperatures were statistically significantly higher ($p < 0.05$) for the devitalized tissues compared to the cryopreserved tissues. The sheep study revealed no significant change ($p < 0.05$) in the dimensions of the devitalized patch relative to the cryopreserved patch. The devitalized tissue recellularized *in vivo* and did not calcify except around the sutures. The infiltrating cells were not apoptotic and stained positively for alpha smooth muscle actin, Factor VIII and Type I collagen mRNA production. In contrast, the cryopreserved tissues had reduced cellularity and calcified; the infiltrating cells were apoptotic and stained weakly for alpha smooth muscle actin, Factor VIII and Type I collagen.

Conclusion: These assessments demonstrate that the devitalization process resulted in tissues with low levels of processing reagent residuals that retain their biomechanical integrity at the macroscopic and molecular level relative to cryopreserved tissues. The sheep model explants indicate that the tissues were able to withstand physiological pressures, recellularize, and resist calcification and apoptosis. The explants further demonstrated that infiltrating cells were biochemically active. Therefore, devitalized tissues represent a safe and viable biohospitable tissue engineered matrix with potential for a wide variety of cardiac and vascular applications.

REFERENCES:
1. U.S Patents 5,843,182; 5,632,778; 5,336,616
2. Simon P, et al. "Early failure of the tissue engineered porcine heart valve Synergraft in pediatric patients." Eur J of Card Thoracic Surgery 2003 Jun; 23 (6):002-6.

THE USE OF ADULT DERIVED ADIPOSE STEM CELL FOR BONE REGENERATION

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Introduction

An adult stem cell (ASC) is an undifferentiated cell found among differentiated cells in a tissue or organ. It can renew itself, and it can differentiate to yield the major specialized cell types of the tissue or organ. The primary roles of (ASC) in a living organism are to maintain and repair the tissue in which they are found. Given the right conditions, some adult stem cells have the ability to differentiate into a number of different cell types. Adult tissues reported to contain stem cells include brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, liver and adipose tissue, or fat.

Adipose is an attractive source of ASC because it is abundantly available, easily accessible and routinely discarded in medical procedures. In vitro, adult-derived adipose stem (ADAS) cells have been induced to form adipose, muscle, cartilage, and bone. Due to their availability and plasticity, many consider that autologous ADAS cells will become the basis of therapies for tissue replacement and regeneration necessary because of injury or disease. The present study demonstrates the use of ADAS cells to form bone tissue.

Methods

Cell culture: Human adult adipose tissue from a fresh surgical specimen was obtained and ADAS cells were isolated and expanded using a hydrogel cell-culture system. Cells were cultured in Dulbecco's modified eagle medium (DMEM) with 10% fetal bovine serum (FBS) and gentamicin.

Cell surface characterization/analysis: ADAS cell verification was completed using fluorescence-activated cell sorting (FACS) analysis. The population of ADAS cells in culture was identified by the presence of surface markers - cellular differentiation (CD) antigens.

Osteogenic differentiation in vitro: ADAS cell cultures were induced towards osteogenic differentiation using osteogenic media (DMEM with 1% FBS, dexamethasone, beta-glycerol phosphate, ascorbic acid) or by exposure to demineralized bone matrix (DBM). Osteogenic differentiation was determined by alkaline phosphatase (ALP) activity. As controls, C2C12 cells were cultured and tested similarly.

Bone formation in vivo: Cultured ADAS cells (1x108/mL) and/or DBM (4 mg/mL) were encapsulated within our hydrogel culture system and injected into subcutaneous site of athymic nude mice. The injected mixtures were harvested at 1, 2, and 4 weeks, and were analyzed to determine osteogenic differentiation using histological methods.

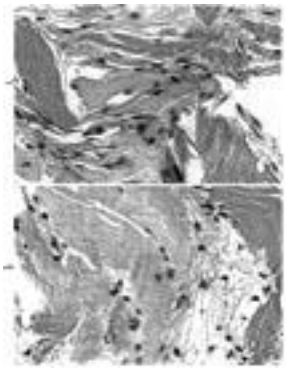
Results

Cell culture: The fibrin-based hydrogel cell-culture system showed excellent biocompatibility in terms of allowing cellular proliferation and migration.

Cell surface characterization/analysis: ADAS cells were identified by the presence of surface antigens, CD34 and CD105 and the absence of surface antigens CD45 and CD31. Osteogenic differentiation in vitro: The ALP activity was more than 1,000-fold elevated in ASCs than C2C12 cells.

Bone formation in vivo: At 1, 2, and 4 weeks post-implantation, greater vascularization was visually evident for the ADAS cells/DBM/hydrogel implant than the DBM/hydrogel. Furthermore, histologic examination of the 4 week implants revealed a higher degree of new bone formation in the ADAS cells/DBM/hydrogel mixture than the DBM/hydrogel mixture (Figure 1).

Figure 1*. Hemotoxylin and Eosin (H&E) stain of (a) DBM/hydrogel and (b) ADAS cell/DBM/hydrogel implants after 4 week implantation. Note the extensive osteoid formation in the ADAS cell-containing implant.*



Conclusions

This study illustrates the potential clinical use of ADAS cells. Human adipose tissue was harvested and ADAS cells were isolated and successfully expanded in culture using a novel cell-culture system. The cells were induced towards osteogenic differentiation *in vitro*, and used to form bone *in vivo*. The use of adult derived autologous stem cells eliminates the potential of rejection, chronic inflammation and disease transmission when using cellular therapies for the treatment of disease or the generation of tissues.

New techniques to isolate and expand adult stem cells for use in combination with biomaterials will accelerate the development of cellular therapies for patients.

POSTERS

STUDENT POSTERS

Biosensor Surface Modifications Using Type I Collagen and Functionalized Gold Nanoparticles
Lisa Wayland Boettcher, University of Missouri

Extracellular Matrix Produced in Tissue-Engineered Corneal Stromal Equivalents
Rachel Crabb, University of Minnesota

Improved Viability of Adipose-Derived Adult Stem Cells After Cryopreservation Using Hyaluronan as a Media Supplement
Isaac Erikson, University of Utah

Poly(ethylene teraphthalate) (pet) and Poly(tetra fluoroethylene) (ptee); Plasma Surface Modification for Coupling Antibodies
Yolanda Escudero, University of Utah

Multi-Technique Comparison of Immobilized and Hybridized Oligonucleotide Surface Density on Commercial Amine-Reactive Microarray Slides
Ping Gong, Colorado State University

Mechanical Effects of Scaffold Densities on Endothelial Cell Network Formation
Maya Harmon, University of Cincinnati

Effect of a Spacer Thiol on the Surface Coverage, Orientation and Hybridization Properties of Mixed DNA Monolayers on Gold
Chi-Ying Lee, University of Washington

Biominetic Peptide-Amphiphiles for Specific Cell-Biomaterial Interactions
Anastasia Mardilovich, University of Minnesota

Stent Evaluation in a Blood Vessel Mimic
Kristin O'Halloran, University of Arizona

Electrospun Composite Nanfibrous Vascular Graft
Saif Pathan, Clemson University

Biophosphonate - Diethylamino Modified Polyurethane Valve Leaflet Replacement in Ovine Model
Andrew L. Rivard, University of Minnesota

Stem Cell Therapy on Swine Acute Heart Infarction Model
Lepeng Zeng, University of Minnesota

Prolonged Release of Stromal Derived Factor-1 for Cardiac Recruitment of Stem Cell by a Novel Pegylated Fibrin Patch
Ge Zhang, University of Minnesota

GENERAL POSTERS

Enhanced Viability of Stored Human Skin Tone
Kristin Fitzpatrick, Bacterin, Inc.

A Nanofibrous Biocomposite Small-Diameter Vascular Graft
Matt Phaeuf, BioSurfaces, Inc.

Abstract
Surfaces in Biomaterials Foundation 2005 Meeting

Lisa Wayland Boettcher
University of Missouri-Columbia
Department of Biological Engineering

Characterization and Diffusion of a Novel 2-D Network Formed Using Type Collagen and Novel Functionalized Gold Nanoparticles

The development of biocompatible coatings for implantable vascular biosensors providing improved long-term function has yet to be perfected. To facilitate the development of possible cellular coatings, an appropriate extracellular matrix (ECM) must be used to encourage desired cellular behavior including adhesion and morphology on the sensor material surface. We are investigating the utilization of uniquely stabilized gold nanoparticles synthesized with [tris(hydroxymethyl)phosphine]-alanine, (THA), as a crosslinking agent for PureCol™ type 1 collagen.
We performed diffusion studies to investigate the movement of -lactalbumin, a protein similar in size to our analytes of interest: troponin I and T. Diffusion was studied across different collagen layers that were dip coated on cellulose membranes. The designed diffusion system consisted of a perfusion chamber from Oligene™ that had an upper and lower circulation chamber separated by the cellulose membrane and connected to a 12-roller digital peristaltic pump from Ismatec™. A known concentration of -lactalbumin labeled with fluoroscein isothiocyanate was pumped through the upper chamber, at a rate to simulate the cardiovascular system, while samples were taken from the lower chamber and scanned with a fluorometer. Results showed that crosslinked collagen fibers had a higher diffusion coefficient (3.8 x 10-5 cm2/s) compared to the collagen (3.0 x 10-5 cm2/s). Various imaging techniques including TEM, FE SEM, and AFM were used to examine the crosslinked and uncrosslinked collagen structures.

Extracellular Matrix Produced in Tissue-Engineered Corneal Stromal Equivalents

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Purpose: The objective of this investigation was to characterize the microstructure of the extracellular matrix as a function of time in culture and matrix geometry.

Methods: Stromal fibroblasts were seeded onto crosslinked collagen sponges and films and cultured for up to five weeks. Analysis of the extracellular matrix and cell phenotype was conducted using scanning electron microscopy, confocal microscopy, and Western blotting.

Surface Characterization: The size and distribution of the extracellular matrix fibrils produced changed according to time in culture and matrix density. The fibril diameter of the initial matrix was 233±30nm according to atomic force microscopy. When cultured on a collagen sponge, stromal fibroblasts produced extracellular matrix fibrils with diameters of 107±20nm initially and 47.5±17nm after one week of culture. When cultured on a collagen film, stromal fibroblasts produced extracellular matrix fibrils 37-62nm in diameter with standard deviations of 21-39nm with no significant differences between weeks. Furthermore, when cultured between two collagen films for one week, the average diameter of stromal fibroblast produced fibrils was 67±47nm and resembled the distribution of fibrils on single films rather than those produced on the three dimensional sponges.

Phenotypic Characterization: For cells cultured on a collagen sponge, Western blotting of cell extracts showed an increase in -smooth muscle actin after one week in culture. However, immunostaining of stromal fibroblasts cultured on the collagen film showed limited expression of -smooth muscle actin.

Conclusions: The geometry and density of the matrix substrate appears to influence the microstructure of the newly synthesized extracellular matrix and cell phenotype. Further studies will be needed to characterize the relationship between these factors.

Keywords: Collagen Scaffolds

Financial Support provided in part by the Biomedical Engineering Institute at the University of Minnesota, Fight for Sight Foundation, American Society of Biomechanics, and the Department of Mechanical Engineering REU program funded by the National Science Foundation.

Conflicts of Interest: None

Improved Viability of Adipose-Derived Adult Stem Cells After Cryopreservation Using Hyaluronan as a Media Supplement

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Introduction

Adipose-derived adult stem (ADAS) cells are becoming an important player in the burgeoning field of human cell therapy. As this field continues to develop, the issues of culture, cryopreservation, and delivery are becoming progressively more important. It is vital that effective serum-free media are available as cellular therapies progress towards delivery in various biomaterials for clinical applications. Hyaluronan (HA) is a well understood and studied molecule that has been used clinically for many years. Studies have shown its effectiveness in cold storage of human skin grafts, creating scaffolds for tissue engineering, in the cryopreservation of spermatozoa and embryos. The objective of this research was to determine if HA could function as an effective serum substitute media supplement for the cryopreservation of ADAS cells.

Methods

ADAS cells were isolated from human tissue that was harvested according to an institutional review board approved protocol. These cells were cultured in Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and gentamicin. One million cells per one milliliter solution were cryopreserved in one of four different solutions: (1) DMEM with 10% dimethyl sulfoxide (DMSO); (2) DMEM with DMSO and 10% FBS; (3) DMEM with DMSO and 1% HA; (4) DMEM with DMSO and 2% HA. After storing in liquid nitrogen for seven days, the cells were rapidly thawed and serially diluted in cold medium.

The viability of the thawed ADAS cells was evaluated by five criteria. In each case, the data is expressed as a percent viability. This value was calculated by the ratio of the post-thaw values to the fresh control values.

The survival rate was determined using the trypan blue exclusion test after the removal of the cryopreservation media. The metabolic activity of the cells was evaluated 2 and 24 hours after plating thawed cells. Metabolic viability was measured using the WST-1 cell proliferation reagent. The attachment rates of the thawed ADAS cells were determined by allowing plated cells 24 hours to attach. Unattached cells were rinsed away with PBS and the remaining cells were trypsinzed and counted. Differences in proliferation rates were recorded by observing the number of days required for cultures to become fully confluent. Finally, the ability of the cells to be induced towards adipocyte differentiation was demonstrated by allowing confluent cultures to incubate in adipogenic induction media for two weeks, at which time the cells were fixed and stained with oil red-O.

Results

The survival rates of the cryopreserved cells varied from 64.8 to 69.6 percent. The cryoprotection solution containing 2% HA yielded cells with the highest metabolic viability (Fig 1). The fresh cell control required ten days to reach full confluence. The thawed ADAS cells from each experimental solution required twelve days. Cells frozen in the HA solutions exhibited attachment rates thirty percent higher than the other cryoprotection solutions (Fig 1). Lipid droplets and vacuoles were identified within the cells in both fresh control cultures and in cultures from each experimental solution.

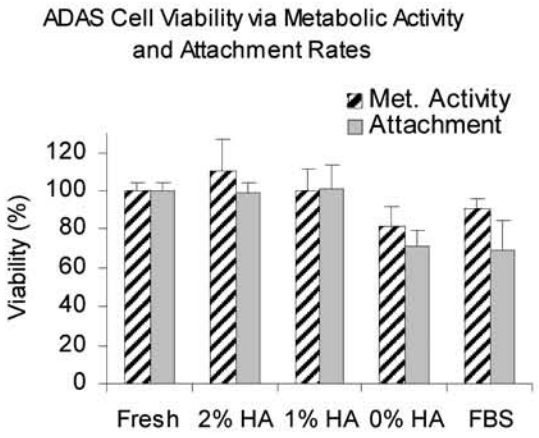

Figure 1

This figure illustrates the data obtained from the WST-1 dye analysis of ADAS cells after cryopreservation. Also shown are the attachment rates of the cells after thawing. "Fresh" represents the fresh unfrozen cells that were the standard of comparison. The 2%, 1%, and 0% HA solutions contain their corresponding concentration of HA and 10% DMSO in DMEM. The "FBS" solution contains 10% FBS and 10% DMSO in DMEM.

Conclusions

These results suggest that HA can function as an effective component of a cryopreservation solution by increasing ADAS cell viability after cryopreservation. Metabolic recovery was accelerated and post-thaw attachment rates were greater when cells were frozen in HA as opposed to the serum-containing positive control ($p<0.05$). ADAS cells frozen in the presence of HA also survived, proliferated, and differentiated at levels equivalent to cells cryopreserved in traditional serum-containing solutions. These findings establish HA as a potential additive for use in the development of a serum-free cryopreservation solution. Optimal media and cryopreservation of stem cells will aid in development of their use in combinational biomaterials.

POLY(ETHYLENE TERAPHTHALATE) (PET) AND POLY(TETRA FLUOROETHYLENE) (PTFE) SURFACE PLASMA MODIFICATION FOR COUPLING ANTIBODIES

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INTRODUCTION

When a polymer is exposed to a plasma many functionalities will be created near the surface. In a typical plasma implantation process, hydrogen is first abstracted from the polymer chain to create radicals at the midpoint of the polymers chains and the polymer radicals then recombine with simple radicals, created by plasma gas, to form oxygen or nitrogen functionalities. Radical species that are created in the plasma zone play an important role in the implantation process. Generally, polymers are hydrophobic, and conversion of these polymers from being hydrophobic to hydrophilic usually improves the adhesion strength, biocompatibility, and other pertinent properties. Formation of oxygen functionalities by ion implantation is one of the most useful and effective processes of surface modification.

METHODS

Selective chemical modifications of polymers are being developed for coupling antibodies. Poly(ethylene terephthalate) (PET) and poly(tetraflouethylene) (PTFE), are widely used in engineering for their bulk properties but they lack of the appropriate surface characteristics for their use in the bioengineering field.[1] Radio Frequency plasma treatment is generally used as a surface modification method which appears to offer a more controllable means than other techniques of chemically modifying PET and PTFE surfaces without affecting bulk properties [2]. We treated the PET and PTFE hydrophobic surfaces with Oxygen and Methacrylic Acid (MAA) radio frequency plasmas in order to create reactive carboxylic acid groups on the surfaces. To activate these groups the samples were treated with adipic dihydrazide. Finally Horseradish Peroxidase antibody was bound to the surface. XPS was used to analyze the surfaces to keep track of the polymer surface modifications for each step.

RESULTS

Table 1 reports the PET and PTFE surface composition, before and after the antibody coupling, expressed in atomic percent.

	PET				PTFE			
	C 1s	O 1s	N 1s		C 1s	O 1s	N 1s	F 1s
a	78.1	21.9	0.0	a	35.4	0.2	n/d	64.4
b	73.5	26.5	0.0	b	37.6	0.7	0.2	61.6
c	68.5	31.4	0.0	c	75.7	10.0	1.0	13.4
d	74.9	17.4	4.7	d	84.4	12.6	2.3	0.8
e	72.3	20.9	5.6	e	68.3	13.4	6.3	11.3

Table 1. XPS PET and PTFE surface composition before and after treatment. a) Untreated. b) O_2 Plasma. c) O_2 and MAA Plasma. d) O_2 and MAA plasma and Adipic Dihydrazide. e) O_2 and MAA plasma, Adipic Dihydrazide and Horseradish Peroxidase.

DISCUSSION

The XPS spectras of treated PET and PTFE reveal the presence of nitrogen-containing groups that indicates the antibody presence.

REFERENCES

1. Hidetoshi K Kumagai, Denbo Hiroki, Nobuyuki Fujii, and Takaomi Kobayaski. (2003) Poly(ethylene terephthalate) decomposition process in oxygen plasma; emission spectroscopic and surface analysis for oxygen-plasma reaction.
2. D.J. Wilson, R.L. Williams, and R.C. Pond. (2001) Surface and Interface Analysis 31: 385-396.

Multi-technique comparison of immobilized and hybridized oligonucleotide surface density on commercial amine-reactive microarray slides

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Abstract:

To establish a quantitative, corroborative understanding of observed correlations between immobilized probe DNA density on microarray surfaces and target hybridization efficiency in biological samples, we have characterized amine-terminated, single-stranded DNA probes attached to amine-reactive commercial microarray slides and complementary DNA target hybridization using fluorescence imaging, X-ray photoelectron spectroscopy (XPS) and ^{32}P-radiometric assays. Importantly, we have reproduced immobilization densities for DNA probes on microarray formats in macroscopic spotted dimensions using high ionic strength, high concentration DNA probe solutions to permit direct XPS surface analysis of DNA-surface chemistry with good reliability and reproducibility. Target capture hybridization efficiency with complementary DNA exhibited an optimum value at intermediate DNA probe immobilization densities. The macroscopic model provides a new platform for the study of DNA surface chemistry using highly sensitive, quantitative surface analytical techniques (e.g., XPS, ToF-SIMS). Sensitive ^{32}P-DNA radiometric measurements can now be directly calibrated with more routine XPS DNA signals, facilitating future routine DNA density determinations without the use of hazardous radioactive assay. The objective is to provide new insight into surface chemistry influences on DNA probe environments that affect the efficiency of target capture in bioassay solutions in order to improve microarray assay performance.

Effects of Scaffold Mechanical Properties on Endothelial Cell Network Formation

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Background. Tissue engineering is an emerging field aimed to create new tissues using cells, biomaterials, biotechnology and medicine. Wound healing is a specific area in which tissue engineering can be used to assist in healing with multiple types of wounds. Angiogenesis (capillary formation) plays an important role in virtually all stages of wound healing, with a variety of angiogenic stimulators and inhibitors regulating blood vessel formation and regression at each stage. In addition to biochemical signals, mechanical interactions between cells and a surrounding extracellular matrix have been shown to play an important role in promoting or inhibiting capillary formation [1]. In wound healing, cells participating in the process may face a considerable variation in extracellular matrix properties, including mechanical stiffness, depending on the tissue and wound type. *Impaired angiogenesis* is associated with many types of abnormal wound healing, including delayed wound healing in diabetics, scar hypertrophy and keloids [2,3]. Therefore, understanding the role of scaffold mechanical properties in capillary growth is important for development of new strategies to enhance wound healing and promote better tissue repair. In this study, we use a self-assembling peptide scaffold and a recently-developed in vitro angiogenesis model [4] to test the hypothesis that *manipulation of scaffold stiffness (by changing the peptide concentration) will affect capillary-like network formation of endothelial cells embedded in a three-dimensional peptide scaffold.*

Methods. Human microvascular endothelial cells (Cascade Biologics) were cultured on gelatin-coated dishes. For experiments, cells (passage 4-7) were stained with CellTracker Green (Molecular Probes) and embedded into 0.5% or 1.5% the self-assembling peptide gel (RADI-16, Zhang et al., 1995) and cultured for up to 2 weeks using Medium 199 (Gibco) with 10% FCS, 5% heparin, and 1% ECGS (Sigma). Grayscale images of the cell networks were taken using a fluorescent microscope. Correlation analysis was performed [4] to determine a correlation length (a parameter characterizing cell clustering and network formation) for each image. Apoptosis was assessed using 5 μm paraffin-embedded cell-scaffold sections and a TUNEL assay (Roche).

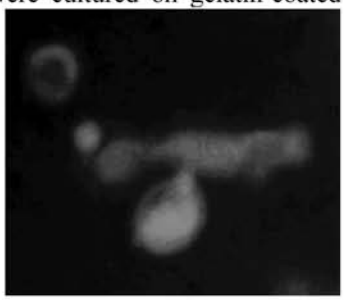

Figure 1. Capillary-like structures formed by endothelial cells at d 3 (40X, 1.5% peptide scaffold).

Results and Discussion. Cell migration and formation of capillary-like structures was observed as early as day 2 after embedding in all peptide concentrations, with lumens clearly visible in most structures (Fig 1). Cells embedded in the more compliant scaffold (0.5% peptide) formed larger clusters consisting of many cells, in contrast to more stiff scaffolds (1.5% peptide), where more elongated single- to five-cell structures were observed. These observations were consistent with significantly higher correlation length for the more compliant scaffold (Fig 2), then the length for the stiffer scaffold. TUNEL assay demonstrated low levels of cell apoptosis (less then 10%) for all peptide concentrations. These results give evidence that scaffold stiffness may affect density and size of newly-formed capillaries and therefore can be a useful tool in regulation of angiogenesis. In addition, fast capillary-like network formation and long-time cell survival demonstrate that this scaffold is promising for future use in tissue engineering applications.

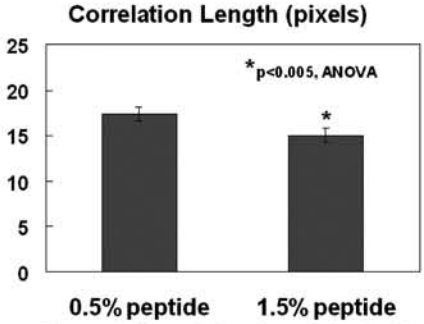

Figure 2. Correlation length for 0.5% peptide samples (average ± SD, n=5, day 4 in culture) was significantly larger than that for 1.5% peptide samples, indicating significantly larger cell networks forming in 0.5% peptide scaffold.

References. 1.Vogel V, Baneyx G. Annu Rev Biomed Eng 2003;5:441-463. 2. Martin A, Komada MR, Sane DC. Med Res Rev 2003;23:117–145. 3. Hausman MR, Rinker BD. Am J Surg. 2004;187(5A):44S-55S. 4. Narmoneva DA, et al. Biomaterials. 2005;26(23):4837-46. 5. Zhang S, et al. Biomaterials. 1995;16:1385-1393.

Effect of a Spacer Thiol on the Surface Coverage, Orientation and Hybridization Properties of Mixed DNA Monolayers on Gold

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DNA surfaces used in biosensors and microarrays require optimum DNA probe coverage and orientation for hybridization as well as a non-fouling background to minimize nonspecific adsorptions. To achieve each of these goals, we evaluated the effect that a spacer thiol [11-mercapto-1-undecanol (MCU)] has on surfaces prepared using singled-stranded DNA with a thiol anchor group (SH-ssDNA). These mixed DNA monolayers have been studied with X-ray photoelectron spectroscopy (XPS), ^{32}P-radiolabeling, near-edge X-ray absorption fine structure spectroscopy (NEXAFS) and surface plasmon resonance (SPR). XPS and radiolabeling results show SH-ssDNA surface coverage decreases with extended exposure to the MCU spacer molecules. NEXAFS indicates increased order and/or changes in the orientation of the SH-ssDNA molecules occurred after only 30 min of MCU backfill. A comparison of hybridization responses from these DNA monolayers was made with a SPR biosensor by exposing the surfaces to complementary DNA in buffer as well as in various concentrations of serum. SPR results indicate that although surfaces with MCU spacer thiols showed resistance towards non-specific DNA binding in buffer, hybridization efficiency is hindered by non-specific serum protein adsorption even at a serum concentration of 1%.

Biomimetic Peptide-Amphiphiles for Specific Cell-Biomaterial Interactions

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The goal of this study is to design peptide-amphiphiles that mimic the cell adhesion domain of the extracellular matrix protein fibronectin. A novel peptide-amphiphile is designed that contains both GRGDSP (Gly-Arg-Gly-Asp-Ser-Pro, the primary recognition site for the $\alpha_5\beta_1$ integrin) and PHSRN (Pro-His-Ser-Arg-Asn, the synergy binding site for $\alpha_5\beta_1$) sequences in a single peptide formulation, separated by a spacer. The goal in designing the spacer is to mimic as closely as possible the distance and the hydrophilicity between the PHSRN and RGD sequences in fibronectin. The effect of the novel peptide on the $\alpha_5\beta_1$ interaction is examined both at the molecular and cellular level.

Bioartificial supported membranes are created from peptide-amphiphile molecules via the Langmuir-Blodgett (LB) technique. LB membranes give the ability to create multi-functional templates and allow control over the peptide surface density, and orientation. LB membranes are characterized with atomic force microscopy (AFM).

At the molecular level, the interaction of pure $\alpha_5\beta_1$ integrins with the peptide-amphiphile is examined by measuring adhesion forces with an AFM. Two different antibodies are used to immobilize and activate isolated $\alpha_5\beta_1$ integrins on the AFM tip. The interaction measured between immobilized $\alpha_5\beta_1$ integrins and the peptide-amphiphile is specific for integrin-peptide binding and is affected by divalent cations in a way that accurately mimics the adhesion function of the $\alpha_5\beta_1$ receptor. The strength of the PHSRN synergistic effect depends on the accessibility of this sequence to $\alpha_5\beta_1$ integrins.

The effect of the PHSRN on the $\alpha_5\beta_1$ interaction is also examined at the cellular level by measuring its impact on the function of human umbilical vein endothelial cells. Blocking studies demonstrate that the fibronectin-mimetic peptide is $\alpha_5\beta_1$ specific. Cells adhere and spread on membranes composed of the novel peptide-amphiphile in a time-dependent manner. Cell adhesion is quantified with a cell proliferation assay based on nucleic acid binding and cell spreading is visualized with confocal microscopy. In vitro studies show that biointerfaces functionalized with the novel peptide-amphiphile show higher adhesion, stronger actin and focal adhesion point formation, and extracellular matrix protein production compared to fibronectin interfaces.

In summary, a novel fibronectin-mimetic peptide-amphiphile is designed that outperforms the fibronectin in terms of cell adhesion, cytoskeletal organization and extracellular matrix protein production. Our novel peptide sequence can find applications in biomaterial functionalization, tissue engineering and targeted drug delivery. Comprehensively, these results demonstrated the value of biomimetic surface science.

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Stent Evaluation in a Blood Vessel Mimic

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Keywords: stents, endothelialization, blood vessel mimic

Introduction

Stent technology is constantly changing as new modification and coating technologies become available. Stents can be drug-eluting, protein or polymer coated, or modified via numerous other methods. Surface treatments are crucial to the cellular response in the vessel, and to the overall success of stent function. Thus, these constantly evolving devices possess great potential, but need to be evaluated before clinical applications are possible. Through the creation of an in vitro blood vessel mimic (BVM), a model system can be established for the initial testing of newly emerging surface modification techniques. Therefore, the purpose of the current work was to utilize in vitro BVMs to evaluate the cellular response to stents.

Materials and Methods

Stent surface modifications were evaluated in an in vitro blood vessel mimic. BVMs were created by pressure-sodding human microvessel endothelial cells onto the lumen of serum-conditioned 3mm I.D. expanded polytetrafluoroethylene vascular grafts (C.R. Bard, Inc.). Following cell sodding, BVMs were cultivated under flow in an in vitro environment in order to establish the cellular lining. After 1 week, stents were deployed into the BVM systems (see Figure 1) via a catheter and introducer port.

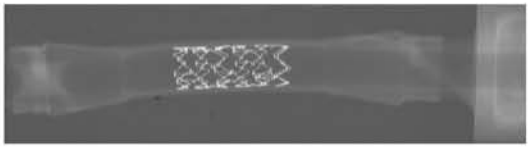

Figure 1: Radiographic image of stented BVM.

Following deployment, flow was continued for 1 week, at which point stented vessels were taken out of the system and fixed in 10% formalin. Vessels were cut longitudinally into three sections. Stent surface analysis was performed using scanning electron microscopy (SEM) to assess cell coverage and cell morphology. In addition, bisbenzimide (BBI) staining of cell nuclei provided information regarding endothelialization of the device surface. Hematoxylin and Eosin (H&E) staining of plastic embedded samples was used in order to assess the strut-associated cell interaction as well as the degree of neointimal thickening.

Results

After 1 week, the BVMs provided a model of stent-induced cellular response. SEM images were used for qualitative assessment of the stent surfaces and of cell morphology. Partial strut coverage was seen. BBI staining was performed to allow quantification of cell coverage. Cell nuclei fluoresced and were counted to calculate cell density on the stent struts. This type of evaluation provided useful information regarding endothelialization of the stent surface (see Figure 2). Hematoxylin and Eosin staining, performed on plastic embedded sections, illustrated the neointimal response to stent implantation.

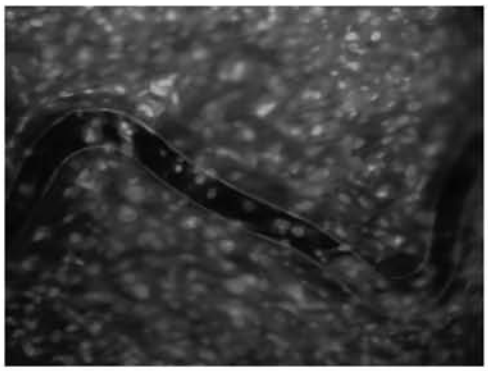

Figure 2: Nuclear stain illustrating cell coverage of stent strut.

Conclusions

Stent surface modifications can be evaluated in an in vitro BVM. Cell coverage of stent surfaces as well as neointimal development can be evaluated, and this analysis provides an assessment of the response of human cells to different surface modifications. The BVM system permits rapid evaluation of stent designs and prototypes and provides a means for initial assessment of stent function prior to the initiation of animal studies.

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ELECTROSPUN COMPOSITE NANOFIBROUS VASCULAR GRAFT

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ABSTRACT

Prosthetic arterial grafts, particularly those of small-diameter (< 5mm internal diameter), continue to suffer high failure rates due to acute thrombosis (clotting). To date, there are no prosthetic vascular grafts accepted for peripheral (below knee) or cardiac bypass. Polyester (PET) and polytetafluroethylene (PTFE) have been used in the fabrication of prosthetic grafts for over fifty years. This work sheds light on a novel technique to produce novel prosthetic grafts using electrospinning technology. This fabrication methodology has distinct advantages over other contemporary methods in that: 1) several polymers can be readily mixed and extruded using solvent system, 2) parameters for nanofibers formation can be controlled, 3) bioactive agents can be synthesized simultaneously with synthetic polymers and 4) devices can be directly fabricated. The goal of this study was to evaluate the effects of electrospinning while combining several different components (PET, polyurethane and Ciprofloxacin). Our hypothesis is that by combining these particular components a novel composite biomaterial with increased biocompatibility (i.e., circumferential compliance, porosity and low water permeability) will be created. The parameters for fabricating these novel fibrous materials will be discussed. Physical, chemical and antimicrobial assays were performed which showed that a novel compliant composite prosthetic material can be synthesized. In addition the produced prosthetic grafts possess infection-resistant properties.

Title: Pathologic evaluation of the polyurethane pulmonary valve leaflet in ovine model

Authors
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ABSTRACT
Background and Aim of Study: Prosthetic heart valves constructed with synthetic polymeric plastics have emerged as an alternative to mechanical and tissue heart valves in clinical use today. In this study we report our findings on a single pulmonary valve leaflet replacement in juvenile sheep using a polyurethane leaflet. The aim of this study is to evaluate the pre-clinical model for functional integrity of the polymeric valve constructs.
Materials and Methods: Four-eight Polypay juvenile sheep of either sex, mean age of 4.9 ± 1.09 months (range: 2.75 to 8.50 months) and mean body weight 40 ± 7.22 kg. (range: 28 to 60 kg) were used in this study done in compliance with NIH standards. In each animal, the anterior pulmonary valve leaflet was excised completely and replaced with a polyurethane leaflet. Laboratory values at 1 and 4 weeks were measured. Implanted leaflets were evaluated at euthanasia for valve failure.
Results: All animals reaching scheduled sacrifice were free of any clinical symptoms (fever, anemia, dehydration, rapid breathing, labored breathing, cyanosis, recumbency, lameness, infection) and deemed to be in good health. Twenty-eight were euthanized at 32 ± 2.99 days, and the remaining twenty at 91 ± 1.14 days. All laboratory lab values at 1 and 4 weeks were within normal age related limits. The heart to body weight ratio was 0.61% (0.17-0.82%). All replacement valves were noted to be structurally intact at the time of pathologic evaluation.
Conclusions: We have developed a single pulmonary leaflet replacement model for juvenile sheep using a sample size three fold greater than has been previously done by Meyers, et al. for this type of valve construct. We believe that the laboratory and pathological results support the ovine pre-clinical model for single pulmonary valve leaflet replacement.

Transplanted Allogeneic Porcine Multi-potent Progenitor Cells (MPCs) in Post-infarcted Pigs Demonstrate Improvement in Heart Function.

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Recent regenerative-medicine approaches using stem/progenitor cells for treatment in cardiovascular disease models have brought varying degrees of success, generating excitement within the scientific community. An ideal cell therapeutic would require a manufacturing process yielding a stable cryopreserved product, suited for use with little or no histocompatibility matching. We have developed a cell expansion platform based on modifications of the MAPC technology and use the term MPC to describe a cell population with broad tissue regenerative capacity.

The present study investigated the effects of allogeneic porcine multi-potent progenitor cells (MPC) against induced-acute myocardial infarction (AMI) in Yorkshire pigs. The pigs were subjected to AMI via LAD (Left anterior descending) artery ligation followed by reperfusion. One hour post AMI, the pigs were treated with direct injections of β-gal expressing MPC (50 million cells delivered in 6 equal injections) in the peri-infarct area. Group 1-control pigs (n=6) received only vehicle, whereas Cohorts 2 (n=5) and 3 (n=7) were injected with MPC in the presence or absence of the cyclosporine A (CsA), respectively. Animals were monitored via Echo, MRI and MRS for 4 weeks before sacrifice. Statistically-significant improvements in cardiac function (Ejection Fraction, Cell Wall Thickness, Circumferential Shortening, CP/ATP ratios) were demonstrated in Groups 2 and 3. Around 0.3-0.5% cell engraftment was observed, with cells frequently found as vessel wall components. No difference in cell retention or cardiac performance was observed in the presence or absence of CsA administration, indicating no allogeneic immune rejection. None of the test pigs developed arrhythmias, indicating cell tolerance without electrical dysfunction. These improvements in heart function could be due to myocardial regeneration, angiogenesis and trophic effects elicited by engrafted MPCs in injured hearts. Thus, direct injection of allogeneic MPCs markedly improved cardiac function in AMI pigs, supporting generation of an off-the-shelf therapeutic for use directly following acute injury.

PROLONGED RELEASE OF STROMAL DERIVED FACTOR-1 FOR CARDIAC RECRUITMENT OF STEM CELL BY A NOVEL PEGYLATED FIBRIN PATCH

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INTRODUCTION: Stromal derived factor 1 (SDF-1) is a key stem cell homing factor that is crucial for mobilizing of stem cells from bone marrow to peripheral blood, and then homing to tissue of diseased organs. It was reported that SDF-1 is transiently over-expressed in ischemic myocardium. Therefore, there is only a limited time window after acute myocardial infarction (AMI) during which stem cells are recruited to injured myocardium for repairing. This study aimed at investigating whether a controlled release of SDF-1 via a novel PEGylated fibrin-patch at the infarcted site would increase the rate of stem cells recruitment and offer potential additional therapeutic benefits. **MATERIALS AND METHODS:** Recombinant mouse SDF-1 was covalently bound to the PEGylated fibrinogen which was evidenced by immunoprecipitation followed by western blot. The PEGylated fibrinogen bounded with the recombinant mouse SDF-1 was mixed with thrombin to form the PEGylated fibrin patch that was patched on the surface of MI. The release kinetics of SDF-1 was detected by ELISA. Using a mouse MI model produced by a ligature on left anterior descending (LAD) coronary, PEGylated fibrin patch bound with SDF-1 (100ng**)** was placed on the surface of the infarcted area of the LV after AMI. The infarcted size, LV ejection fraction (EF by Echo), as well as the percentage of Sca-1^{+}/ C-kit $^{+}$ cells (FACS analysis) within the infarcted area were measured at day 7, 14 and 28 after AMI. **RESULTS:** *In vitro* results showed**:** SDF-1 was successfully bound to the PEGylated fibrin patch and released SDF-1 constantly for up to 10 days. Two weeks after infarction, the myocardial recruitment of C-kit $^{+}$ cells was significantly higher in SDF-1 PEGylated fibrin patch treated group (PEG, n=9) than the AMI group (n=10) ($p<0.05$; $11.20 \pm 1.71\%$ vs. $4.22 \pm 0.96\%$, respectively). At day 28 post AMI, unlike AMI group that SDF-1 recruitment effect disappeared early, the PEG group continued stable releasing of SDF-1 which was accompanied by more stem cell homing. In addition, the LV EF was significantly higher in PEG than the AMI. **CONCLUSION:** These data demonstrate that the PEGylated fibrin patch based delivery of SDF-1 at the infarcted site can improve the rate of stem cell homing and improve LV function in hearts with postinfarction LV remodeling.

Enhanced Viability of Stored Human Skin Tissue

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Introduction

Cadaveric allograft skin is currently the ideal choice for temporary wound coverage for burn victims. Skin is used as a screening method for delivery of pharmaceuticals, and is a source of cells used in various models of bioengineering. However, the viability of transplanted or research skin is compromised when cultured more than 96 hours, and when it is cryopreserved. Extension of the shelf life of cadaveric skin would provide increase availability of cold stored tissue. Depletion of extracellular matrix (ECM) components during storage is believed to play a role in the rapid loss of viability. A novel method was developed to prolong the viability of the skin by supplementing currently used storage media with ECM glycosaminoglycans, hyaluronic acid (HA) and chondroitin sulfate (CS).

Methods

Split-thickness human cadaveric skin was obtained and placed in RPMI-1640 at 4°C. Within 24 hours, the skin was cut into 6 mm pieces and immediately placed in 50 mL conical tubes, each containing 20 mL of either the control or supplemented media. The control medium was RPMI-1640 with 0.05 mg/mL gentamicin. The supplemented media consisted of the control media with 0.5 mg/ml each HA (1700 kDA and CS (shark) combined. The samples were stored at 4°C and the experiment was performed under sterile conditions.

An *in vitro* viability assessment was conducted using the Orion Dissolved Oxygen Probe (Thermo Orion Beverly, MA). For each test, a sample of cultured skin was placed in media contained a Teflon cartridge, epidermal side down. The probe was inserted into the cartridge and a reading taken continuously every ten seconds for 2000 seconds. Oxygen consumption values were calculated by taking the difference of the medium alone and medium with skin dissolved oxygen (mg/L) at each time point; these values were averaged for the duration of the experiment. Significant differences were determined at $p<0.05$.

To assess *in vivo* function, athymic nude female mice were given a split thickness cadaveric skin xenograft, diameter 6 mm. The skin was stored for 7 days prior to transplantation in either control or supplemented media. The transplanted grafts were secured with tegaderm and bandaged. After 1 week, the graft area was excised for histological assessment.

Results: Figure 1. Average Oxygen Consumption

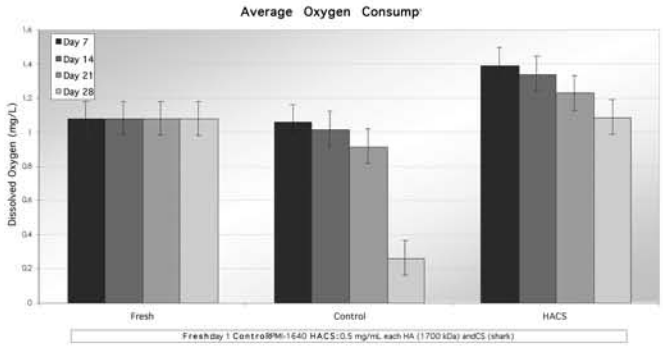

Figure 1: A higher concentration indicates the skin requires and is consuming more oxygen and is more metabolically active. The decrease of the control concentration from day 21 to day 28 suggests greatly decreased viability ($p<0.01$). However, the dissolved O_2 concentrations of the supplemented media remain reasonably constant, with the combined HA/CS solution closest to that of the newly harvested skin (fresh group).

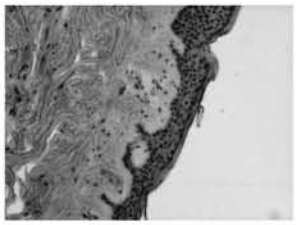

Figure 2: The histological assessment of the grafted skin showed better engraftment with the supplemented skin at day 7. Controls showed separation of keratinized cell layer, indicative of early stages of degeneration (not shown). Fig. 2 shows that the dermal/ epidermal junction was conserved through the graft width in the supplemented grafts. Presence of blood vessels indicated graft vascularization, a positive sign of healing. Migration of host cells into the host dermis at the graft/host interface was evident and signs of inflammatory response suggest healing and the grafted human tissue was in good condition, well maintained and accepted.

Conclusion

Combining the individual biological benefits of HA and CS achieved enhanced cadaveric skin viability to 28 days post-harvest. A higher quality engraftment was seen with supplemented skin.

New approaches to ensure viability and function of tissues and cells will aid the development of biomaterials containing or interfaced with living cells and tissues.

A Nanofibrous Biocomposite Small-Diameter Vascular Graft

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Abstract Summary

There is no small-diameter (< 5mm internal diameter) vascular prosthesis clinically available that is capable of emulating the biological and physical properties of the normal arterial wall. Development of an "off-the-shelf" small vessel prosthesis which emulates some of the natural biologic processes of normal arterial walls would greatly expand the surgical options in treating both peripheral vascular disease as well as coronary artery disease. The goal of this study is to develop *in vitro* a novel nanofibrous bioactive small-diameter (4mm internal diameter) prosthetic vascular graft using electrospinning technology.

Experimental Methods

Electrospinning Methodology: A novel composite Dacron/Type IV collagen graft (ESDC) was prepared using proprietary electrospinning technology.

Water Permeation Assessment: ESDC grafts were cut open in the longitudinal direction and cut into circular segments (15mm diameter, n = 5 segments). A cylindrical reservoir, with a direct water inlet to keep pressure constant, was set to a height of 165mm to the top of the water column. A 5/16" Tight Right fitting (attached to 3/16" tubing) was used to hold the test segment (area = 0.38cm^2) in place without applying stretch to the material. Each ESDC graft piece was pre-soaked in the apparatus for 1 minute in order to de-gas the material, followed by exposure to 120 mm Hg pressure. Volume was collected in a graduated cylinder for 60 seconds and used to determine ml/min/cm^2. Knitted Dacron grafts as well as collagen-sealed Dacron grafts were used as controls.

Determination of Tensile Strength: ESDC graft segments (7mm width, 3cm length; n=3/test condition) were measured and cut. A Q-Test Tensile Strength Apparatus (MTS Systems, Cary, NC) was calibrated according to manufacturer's specifications in a climate-controlled environment. Segment stretching (crosshead speed = 50mm/min, gauge length = 2cm, load cell = 25 lb) was then initiated and terminated upon segment breakage.

Covalent Linkage of rHir/VEGF: ESDC grafts were cut into 0.5cm length segments and weighed (n=4 segments/test group, 2 groups). A stock 50mM sodium bicarbonate buffer solution (pH 8.5) was utilized. A 20mg/ml solution of Traut's reagent was prepared in the bicarbonate buffer and 2ml was added to one set of ESDC segments in 5ml borosilicate glass test tubes. To the other set, 2ml of bicarbonate buffer was added. All segment sets were then reacted for 1 hour on the inversion mixer at room temperature. A 431μM ^{125}I-rHir solution was reacted with sulfo-SMCC in a 1:2 molar ratio. Simultaneously, a 23.8μM ^{131}I-VEGF was reacted with sulfo-SMCC in a 1:2 molar ratio. Both proteins were reacted for 20 minutes at 37°C followed by purification via gel filtration. The ^{125}I-rHir-SMCC solution was brought to a final concentration of 71.8μM and ^{131}I-VEGF-SMCC was brought to a final concentration of 4.76μM. ^{131}I-VEGF-SH (2ml) was exposed first to the control and test ESDC segments followed immediately by addition of 2ml of ^{125}I-rHir-SMCC. These segments were incubated for 3 hours at room temperature on an inversion mixer. Segments were then removed, followed by washing and sonication for 5 minutes (X 4). Utilizing the specific activity, the amount of ^{125}I-rHir and ^{131}I-VEGF bound (ng) per weight of graft segment (mg) was determined.

Antithrombin Activity by Immobilized rHir: ESDC graft segments with non-specifically and covalently bound ^{125}I-rHir were examined for antithrombin activity using a chromogenic assay for thrombin S-2238. Thrombin concentrations of 1, 2.5, 5 and 10 NIHU were evaluated. The assay was started by the addition of 1ml of 100μM S-2238 with the change in absorbance per minute monitored at 15-second intervals for 3 minutes at 410nm.

EC Stimulation by Immobilized VEGF: Circular segments of nanofibrous Dacron, ESDC with no modification and ESDC with covalently bound rHir/VEGF were utilized (n=4 segments/test group). All sets of segments were placed into sterile PBS containing 20% pen-strep antibiotic for 24 hours prior to use. Trypsinized HUVEC's (0.5ml) were added to each well. Cells were allowed to incubate overnight in a 37°C incubator with 5% CO_2. The next day, the F12K medium was suctioned off and 1.5ml of a 10% Alamar Blue dye (in F12K medium with 10% FBS) added, an assay with which our group has extensive experience. Alamar Blue was also added to 4 blank wells to serve as a negative control. This solution was incubated for 4 hours followed by removal of 1ml into clear-sided fluorescence cuvettes. Solutions were then diluted 1:1 with F12K containing 10% FBS and fluorescence measured for each sample using a Perkin-Elmer LS-5B spectrofluorometer (excitation – 506nm, emission - 590nm, slit width – 5). Difference in fluorescence intensity between the blank and segment-containing wells was determined.

Results

Using electrospinning technology, a novel nanofibrous small-diameter graft was constructed. This construct had a consistent 4mm internal diameter throughout the lumen of the graft (graft length = 7cm). Within the graft wall, two independent fibers were evident. Collagen nanofibers were present throughout the material and were approximately 10-fold smaller than the Dacron fibers. These smaller fibers were not present when Dacron was electrospun alone. Formation of the collagen fibers within the ESDC grafts was confirmed via acid red uptake, which was significantly greater in the ESDC grafts than the Dacron electrospun controls alone. Water permeation for the ESDC grafts was 29 ± 11 ml/min/cm^2, a result that was significantly below the 100 ml/min/cm^2 threshold determined to prevent blood seepage through the graft wall. Covalent linkage of ^{125}I-rHir to the ESDC surface (590 ± 103 ng/mg) was 7.7 fold greater than the controls (77 ± 53 ng/mg, $p < 0.0001$). Covalent immobilization of ^{131}I-VEGF (108 ± 38 ng/mg) was also significantly greater (4.5 fold) than the control ESDC segments (24 ± 10 ng/mg, $p < 0.006$). ESDC segments with covalently bound ^{125}I-rHir inhibited significantly greater amounts (2 – 5 fold greater inhibition, $p < 0.05$) of thrombin as compared to segments with non-specifically bound ^{125}I-rHir. Lastly, the ESDC-rHir-VEGF segments had consistently greater HUVEC growth as indicated by fluorescence as compared to the ESDC and nanofibrous Dacron segments.

Conclusions

A novel nanofibrous biocomposite small-diameter vascular graft was constructed. This novel graft has excellent kink-resistance as well as flexibility to bend due to the wall construction. Two independent fibers (Dacron and collagen) are present within the graft wall as indicated via SEM as well as by dye uptake. Physical and chemical properties of the ESDC grafts were characterized. A biologically-active surface was created via "targeted" protein immobilization to the collagen fibers in order to incorporate specific functions within the graft matrix.

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Keywords

Prosthetic Vascular Grafts, Nanotechnology, Polyester, Collagen

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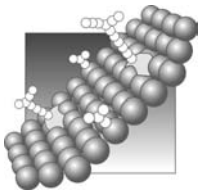

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