



6TH annual
California
Tissue
Engineering
Meeting

University of California, Los Angeles

November 30 & December 1

Dear Colleagues:

Welcome back to UCLA, home of the Inaugural California Tissue Engineering Meeting. We are excited to host this year's joint meeting of the sixth annual California Tissue Engineering Meeting, the Los Angeles Tissue Engineering Initiative, and the California Triple Helix in Regenerative Medicine.

In keeping with past CalTEM meetings, this year will also feature three scientific sessions on the topics of Biomaterials Engineering, Stem Cell Biology and Tissue Engineering, and Cytokines & Cell Signaling.

This year's keynote lecture will be delivered by Prof. Hong Wu from the UCLA Center of Regenerative Medicine and Stem Cell Research. Prof. Wu is world renowned for elucidating the molecular mechanism of PTEN controlled stem cell self-renewal, proliferation, and survival, as well as its roles in controlling "cancer stem cells" and tumorigenesis. Dr. Wu will discuss her recent finding on PTEN's role in controlling neural stem cells and neurogenesis. The keynote lecture is followed by lunch and poster presentations, which allow ample opportunities for networking and informal questions.

We have added a new session to explore the role that the California Triple Helix: government, industry, and universities, can play to leverage the state's emerging growth in stem cell research and its impact on regenerative medicine. The California Triple Helix in Regenerative Medicine session focuses on the vital, intertwining challenges and opportunities that are best addressed collaboratively by government, industry, and academia stake holders. This session will feature focused talks followed by an open discussion panel to address the relevant issues. Our distinguished panelists will bring distinct perspectives from UC practices on intellectual properties, academic licensing, industry, venture capital, and start-up companies that are bringing exciting new products to the area of Regenerative Medicine.

I would like to thank the Scientific Advisory Committee for their thoughtful suggestions, and the sponsors for their generous donations. Special thanks to the volunteer staff and students from UCLA Bioengineering, who have worked for several months to put together an exciting and focused agenda bringing together experts and visionaries in our field. We hope you will have a productive few days.

Regards,
Ben Wu, DDS, PhD
Chair, Organizing Committee

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Gregory Evans
UC Irvine

Michael Longaker
Stanford University

Anthony Ratcliffe
Synthasome

Robert Sah
UC San Diego

Warren Garner
USC

Jonathon Mansbridge
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**Joint Meeting of the
California Triple Helix in Regenerative Medicine
6th Annual California Tissue Engineering Meeting**
November 30 - December 1, 2007
Ackerman Grand Ballroom, UCLA

FRIDAY, NOVEMBER 30, 2007

9:00 am *Registration & Poster Setup – Continental Breakfast*

9:30 am *Opening Remarks*
Benjamin Wu, D.D.S, Ph.D.
Associate Professor & Vice Chair, UCLA, Department of Bioengineering

Session 1: Biomaterials Engineering

Moderators: Andrea Kasko, Assistant Professor, Department of Bioengineering, UCLA
Heather Maynard, Assistant Professor, Department of Chemistry & Biochemistry, UCLA

9:35 am *Synthesis and Characterization of Enzymatically Crosslinked Protein Polymer Hydrogels for Tissue Engineering Applications*
Annalise Barron, Ph.D., Stanford University, Department of Bioengineering

10:00 am *Designing a Matrix to Recreate the Optimal Microenvironment for a Tissue Engineered Cornea*
Marc Davidson, et al., Harvey Mudd College, Department of Engineering

10:15 am *Magnetically Actuable Scaffolds for Tissue Regeneration*
Julia Mack, et al., Teledyne Scientific, Thousand Oaks, California, USA

10:30 am *Biosurface Engineering*
Karen Christman, Ph.D., UC San Diego, Department of Bioengineering

10:55 am *Electrospun Polycaprolactone Nanofibrous Scaffold for Liver Tissue Engineering*
Jessica Gluck, et al., Current UCLA Bioengineering Graduate Student
(College of Textiles, North Carolina State University, Raleigh, NC)

KEYNOTE SPEAKER

11:10 am *Hong Wu, M.D., Ph.D.*
UCLA, Department of Molecular & Medical Pharmacology
Center of Regenerative Medicine and Stem Cell Research

LUNCH & POSTER SESSION

Session 2: The California Triple Helix in Regenerative Medicine

Moderators: Anthony Ratcliffe, President & CEO, Synthasome, Inc.
Timothy Deming, Professor & Chair, Department of Bioengineering, UCLA

1:00 pm *Transferring Novel Tissue Engineering and Regenerative Medicine Discoveries from the UC to the Public*
Kathryn Atchison, D.D.S., M.P.H., Vice Provost of Intellectual Property & Industry Relations and Associate Vice Chancellor for Research, UCLA

1:25 pm *The Tissue Engineering Divide: An Explosion in Discovery, An Industry in Peril*
Dawn Applegate, Ph.D., President and CEO, RegeneMed

1:50 pm *Advancing Theregen's Cell-based Epicardial Patch Through the Evolving Regulatory and Public Policy Environment*
Mike Siani Rose, Founder & President, Theregen

2:15 pm **COFFEE BREAK**

2:30 pm *Panel Discussion*
Moderators:
 Anthony Ratcliffe, President & CEO, Synthasome Inc.
 Benjamin Wu, Associate Professor & Vice Chair, Department of Bioengineering, UCLA
Panel Members:
 Kathryn Atchison, D.D.S., M.P.H., Vice Provost of Intellectual Property & Industry Relations and Associate Vice Chancellor for Research, UCLA
 Dawn Applegate, Ph.D., President and CEO, RegeneMed
 Mike Siani Rose, Founder & President, Theregen
 Sharon Stevenson, D.V.M., Ph.D., Co-Founder and Managing Director, Okapi Venture Capital

4:00 pm **RECEPTION – 5101 ENGINEERING V**
 (New home to Bioengineering – 4th & 5th Floors)

SATURDAY, DECEMBER 1, 2007

8:00 am *Late Registration/Continental Breakfast*

Session 3: Stem Cell Biology & Tissue Engineering

Moderators: James Dunn, Associate Professor, Department of Bioengineering, Department of Surgery, Division of Pediatric Surgery, UCLA
 Suzie Riley, Senior Manager, Development, Cytori Therapeutics

8:30 am *Using Biomaterials in Differentiation Strategies with Human Embryonic Stem Cells*
 Amander Clark, Ph.D., UC Los Angeles, Department of Molecular, Cell & Developmental Biology

8:55 am *Mesenchymal Stem Cells Differentiation into Osteoblasts on 2D and in 3D Fibrin Scaffolds*
 Melissa Chow, et al., UC Los Angeles, Department of Bioengineering

9:10 am *In Vivo Cartilage Regeneration using Human Embryonic Stem Cell-derived MSCs*
 Nathaniel Hwang, et al., UC San Diego, Department of Biomedical Engineering

9:25 am *Spinal ischemic paraplegia: modulation by stem cell implant*
 Martin Marsala, M.D., UC San Diego, Department of Anesthesiology, Cancer Symptom Control Program

9:50 am *Characterization and Transplantation of Neural Crest Progenitor Cells*
 Carrie Geisbauer et al., UC Los Angeles, Department of Bioengineering

10:05 am **COFFEE BREAK**

Session 4: Cytokines & Cell Signaling

Moderators: Robert Sah, Professor & Vice Chair, Department of Bioengineering, UCSD
 Shyni Varghese, Assistant Professor, Department of Bioengineering, UCSD

10:20 am *Using Tissue Engineering to Study Cytokine Function*
 Manuela Martins-Green, Ph.D., UC Riverside, Department of Cell Biology & Neuroscience

10:45 am *In vitro Models to Study Myofibroblast Activation*
 Ruth Baxter, et al., UC Los Angeles, Department of Orthopaedic Surgery

11:00 am *Mechanotransduction of Chronic Neural Injury Via Schwann Cells*
 Ranjan Gupta, M.D., Ph.D., UC Irvine, Department of Orthopedic Surgery

11:25 am *Spatial Dynamics of Epithelial Population Growth*
 Jin-Hong Kim, et al., California Institute of Technology, Division of Engineering and Applied Science and Division of Chemistry and Chemical Engineering

11:40 am *Controlling Cartilage Shear and Sliding in vitro for Mechanobiology and Tissue Engineering*
 Nguyen QT, et al., UC San Diego, Department of Bioengineering

12:00 pm **CLOSING REMARKS/AWARDS**

Hong Wu, Ph.D., M.D.

Dr. Hong Wu is a Professor of Molecular and Medical Pharmacology, and a member of UCLA Molecular Biology Institute and Jonsson Comprehensive Cancer Center. She is the Co-Associate Director of Genitourinary Oncology Program Area of UCLA Jonsson Comprehensive Center, co-Director of Cancer Stem Cell Program area of the Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research and the Director of the UCLA ES Cell and Transgenic Mice Shared Resource. She serves as a member of the NCI Mouse Model of Human Cancer Consortium Steering Committee. A major research focus of Dr. Wu's laboratory is to study the molecular mechanism of PTEN controlled tumorigenesis. By generating tissue-specific PTEN deficient animal models, including prostate cancer model, Dr. Wu's laboratory elucidated the important role of PTEN in regulating stem cell self-renewal, proliferation, and survival, as well as its roles in controlling "cancer stem cells". These models have been used for pre-clinical studies of new therapeutic agents and for identifying biomarkers for human prostate cancers.

In this lecture, Dr. Wu will discuss their recent finding on PTEN's role in controlling neural stem cells and neurogenesis.



BIOGRAPHIES

CTEM Organizing Committee and Moderators

Kathryn A. Atchison, D.D.S., M.P.H.

Kathryn A. Atchison, D.D.S., M.P.H., is Vice Provost of Intellectual Property and Industry Relations at UCLA. Since assuming the position in 2005, she has transformed the Office of Intellectual Property (OIP) by improving open communication with and education for faculty and graduate students, initiating a student internship program, and moving industry contracting into a newly revamped office, the Office of Intellectual Property and Industry Sponsored Research, to enable better service to the campus and industry alike.

In her role, Atchison helps strengthen UCLA's existing industry relationships and fosters new ones, with an eye toward making the products of UCLA's research programs available, and thereby enhancing economic development in the local region, state, and nation. She serves as a Board member for Entreetech, an organization designed to serve entrepreneurs in the Los Angeles area.

Academically, Atchison is a Professor in the UCLA School of Dentistry and the UCLA School of Public Health. She served as President of the American Association of Public Health Dentistry (AAPHD) from 2006 – 2007, and now is Immediate Past President. As President of AAPHD, Atchison established the ongoing collaboration with professional organizations in dentistry to seek funding to develop the methods and metrics for conducting evaluations of workforce demonstrations.

Atchison is a senior dental health services researcher who studies quality and effectiveness of health services. Her interest in disparities in health research began when she served as a Staff Dentist at a neighborhood health center in Boston's Chinatown. She has had substantial experience conducting and leading collaborative multidisciplinary, community-based research. She co-led the 1997 NIDCR-sponsored workshop "Challenges for Oral Health Promotion Research". She has published extensively on outcomes assessment and quality of care issues, such as perceptions of oral health and development and evaluation of psychosocial outcome measures, for which she developed the Geriatric/ General Oral Health Assessment Index, a quality of life measure used in many countries.

In 2001 she launched the implementation of a state-of-the-art integrated management information system for the School of Dentistry that encompasses an electronic patient dental record (EPR) and an academic management system that tracks the characteristics and activities of dental providers. Dr. Atchison was an invited speaker on the NIDCR-sponsored conference on "Dental Informatics and Dental Research". Atchison serves as the Principal Investigator of an RO1: "Patient preferences for Treatment of Mandibular Fracture", is PI for an R03 "Health Literacy" study in which she is examining the effect of culture and health literacy on accessing preventive services in a dental clinic, and is an investigator on a multi-year evaluation program for the Robert Wood Johnson's Pipeline, Prevention and Practice program to improve dental education.

Dawn Applegate, Ph.D.

Dawn R. Applegate, Ph.D. is President and CEO of RegeneMed, Inc., a tissue engineering company focused on application of tissue-based products to drug discovery, diagnostics, biosensors, medical devices and implants. She obtained her Ph.D. from MIT in Chemical Engineering. For the past 15 years she has led the development of first-of-a-kind, FDA-approved cGMP tissue engineered manufacturing systems at Advanced Tissue Sciences and RegeneMed for which she holds two patents, and participated in regulatory submissions, clinical trials and marketing efforts related to these systems. In her last role as Director of Technology Development at Advanced Tissue Sciences, she conceived, developed and attracted \$6M in venture funding and \$8M in NIH SBIR grants to the RegeneMed spinout business. RegeneMed's first product, liver tissue co-cultures, will replace cell-based tests, providing a more physiologically relevant, human predictive system to address the leading cause of drug failures in clinical trials, poor drug metabolism and toxicity.

Annelise Barron, Ph.D.

Dr. Barron received her Ph.D. in chemical engineering in 1995 from the University of California, Berkeley, and a B.S. cum laude in chemical engineering in 1990 from the University of Washington in Seattle. She was an NIH-NRSA postdoctoral fellow in pharmaceutical chemistry at the University of California, San Francisco. She joined the faculty at Northwestern University in 1997, and moved to Stanford in 2007. She has won several awards for scholarship, including the Presidential Early Career Award for Scientists and Engineers, the Beckman Young Investigator Award, and the Camille and Henry Dreyfus Teacher-Scholar Award. She has authored more than 70 peer-reviewed research publications. Dr. Barron is presently a member of the Advisory Council to the NIH Director, Dr. Elias Zerhouni, and a permanent member of the NIH's Synthetic and Biological Chemistry Study Section.

Dr. Barron directs a group of 29 Ph.D. students and postdocs involved in diverse research projects at the interface of biotechnology, polymer science, and medicine. About half of her group's research has been focused on developing novel materials and strategies for high-throughput DNA sequencing and genotyping by microchip electrophoresis, including technologies for the rapid detection of genetic mutations related to cancer. The other half of her group creates and studies novel, stable peptide analogs that mimic bioactive protein domains and have promising therapeutic applications.

Karen Christman, Ph.D.

Dr. Karen L. Christman received her B.S. in Biomedical Engineering from Northwestern University in 2000. She then moved to California for both her graduate and postdoctoral work, receiving her Ph.D. from the University of California San Francisco and Berkeley Joint Bioengineering Graduate Group in 2003 under the direction of Dr. Randall J. Lee at UCSF. She completed her postdoc work in the lab of Dr. Heather D. Maynard at the University of California, Los Angeles in the fields of polymer chemistry and nanotechnology. She is currently an Assistant Professor in the Department of Bioengineering at the University of California, San Diego and is interested in applying polymer chemistry and nanotechnology techniques to the treatment and regeneration of cardiovascular tissues both in vivo and in vitro.

Amander Clark, Ph.D.

Amander Clark is amongst the first group of highly touted young stem cell Scientists to be recruited to UCLA. Dr Clark is a member of the Department of Molecular Cell and Developmental Biology, Jonsson Comprehensive Cancer Center, Molecular Biology Institute and the Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research. Dr. Clark's main research program is to understand the fundamental molecular mechanisms that regulate early human embryo development. Prior to joining the faculty at UCLA, Dr. Clark was on faculty at the University of California, San Francisco where she worked in the laboratory of Dr. Renee Reijo Pera and built a novel cell based model to study human embryo development using human embryonic stem cells. Dr. Clark's work was the first to demonstrate that human embryonic stem cells could be used to differentiate the early embryonic stages of the human germ line. Germ cells ultimately form our gametes (sperms and eggs), which are essential for fertility and subsequent normal embryo development. Dr. Clark's lab explores embryonic germ cell formation in order to examine mechanisms that lead to infertility, germ cell cancers, fetal demise, infant mortality and birth defects. Therefore, Dr. Clark's lab is amongst the first to use embryonic stem cells to advance a new key area of research in reproductive medicine. Dr. Clark obtained her B.Sc with Honors and PhD from the University of Melbourne, Australia. Dr Clark has been the recipient of a number of awards for her stem cell research including the inaugural Young Investigator Award from the International Society for Stem Cell Research, and a Career Development award from the STOP CANCER foundation. Dr. Clark's scientific work has also been published in Nature, Science, Human Molecular Genetics, Stem Cells and Cancer.

Timothy J. Deming, Ph.D.

Timothy J. Deming received a B.S. in Chemistry from the University of California, Irvine in 1989, and graduated with a Ph.D. in Chemistry from the University of California, Berkeley, under Bruce Novak in 1993. After a NIH postdoctoral fellowship at the University of Massachusetts, Amherst with David Tirrell, he joined the faculty in the Materials Department at the University of California, Santa Barbara in 1995. Here he held a joint appointment in the Materials and Chemistry Departments where he was promoted to Associate Professor in 1999 and Full Professor in 2003. His appointment is now as the Chair of the Bioengineering Department at the University of California, Los Angeles. Current research interests include polypeptide synthesis, self-assembly of block copolypeptides, and biological activity of polypeptides, for which he has received young investigator awards from the National Science Foundation, the Office of Naval Research, The Arnold and Mabel Beckman Foundation, the Alfred P. Sloan Foundation, the Camille and Henry Dreyfus Foundation, the Materials Research Society, and the IUPAC Macromolecular Division.

James Dunn, M.D., Ph.D.

James Dunn obtained a B.S. degree in Biology and Chemical Engineering from the California Institute of Technology. He then completed the M.D./Ph.D. program in the Division of Health Sciences and Technology at Harvard Medical School and Massachusetts Institute of Technology. Subsequently, he trained in general surgery at the UCLA School of Medicine and in pediatric surgery at the Riley Hospital for Children. Dr. Dunn is currently an Associate Professor in the Departments of Bioengineering and Surgery at the University of California, Los Angeles. Dr. Dunn's research interests are in the area of tissue engineering of internal organs, including the intestine, the liver, and the adrenal cortex.

Gregory Evans, M.D., FACS

Dr. Gregory Evans, MD, FACS is Professor of Surgery and Biomedical Engineering and Chief of the Aesthetic and Plastic Surgery Institute. He is a medical graduate of the University of Southern California and completed his plastic surgery residency at The Johns Hopkins Hospital.

Prior to coming to UCI, Dr. Evans spent seven years performing cancer reconstruction procedures, including free tissue transfer for head, neck and breast at the M.D. Anderson Cancer Center in Houston, Texas. Dr. Evans specializes in the full spectrum of plastic surgery, with a focus on cosmetic surgery such as face, eyes, abdomen, breasts and body contouring. He continues to provide state of the art cancer reconstruction for breasts, head and neck, and extremities.

As a physician scientist, he is also a leading researcher in tissue engineering. Research projects have included recreation of new tissue equivalents for damaged nerves that will help restore function to cancer, burn and trauma patients.

Dr. Evans is certified by the American Board of Plastic Surgeons and is currently a director for the American Board of Plastic Surgery.

Warren Garner, Ph.D.

Dr. Garner is an Associate Professor of Surgery at the Division of Plastic and Reconstructive Surgery at the University of Southern California School of Medicine. He is a Board Certified Plastic Surgeon and Fellow of the American College of Surgeons. Dr. Garner is a burn/reconstructive surgery specialist, a specialist in wound care and management, and a researcher of skin healing and burn wounds.

He performed his undergraduate work and completed medical school at the University of Kansas, in Kansas City, Missouri after which he completed a general surgery residency at Ohio State University. Dr. Garner also completed a fellowship in Critical Care at Ohio State University. His Plastic Surgery training took place at the prestigious Washington University School of Medicine in St. Louis, Missouri.

Dr. Garner is a staff member at several hospitals including 1) USC University Hospital, 2) Los Angeles County + USC Medical Center, 3) Kenneth Norris Hospital, 4) Rancho Los Amigos National Rehabilitation Center, and 5) Kaiser Permanente Hospital. He is involved in burn injury, wound healing, fibrosis, cytokines, and inflammation research and is currently serving as the Director of the LAC + USC County Burn Unit. As an Associate Professor of Surgery and as Director of the Burn Unit, Dr. Garner heads the Burn Team and manages the 4 month Burn Surgery and Reconstruction training rotation of USC Plastic Surgery Residents.

Dr. Garner also is involved in numerous national organizations including the American Burn Association, the Wound Healing Society, the American Society of Plastic and Reconstructive Surgery, the American Association of Plastic Surgeons, the Plastic Surgery Educational Foundation, the International Society for Burn Injuries, the Plastic Surgery Research Council, the Society of Critical Care Medicine, and the Society for Investigative Dermatology. He has served on and chaired committees, as well as served in leadership positions within most of these organizations. He presently serves on the board of directors of the Wound Healing Society, a society aimed at advancing the science and practice of wound healing, as their Treasurer. In conjunction with his research and private practice, Dr. Garner has written and co-authored numerous articles, chapters, and abstracts for various medical journals. In 1990 he received the Outstanding Abstract Award from the American Burn Association.

Dr. Garner is an outdoorsman and during his leisure time, when he is not teaching, researching, seeing patients or performing surgery, he enjoys freshwater and saltwater fishing. He is an avid connoisseur of fine wine as well.

Nathalie Gosset

Nathalie Gosset is Head of Business Development and Marketing at the Alfred Mann Institute at the University of Southern California. She created and implemented this new function at AMI after leading the Engineering Department at the Institute for several years. The Alfred Mann Institute is a non-profit acceleration and incubation center for commercialization of biomedical inventions from USC.

Ms. Gosset has over 20 years experience in the development and commercialization of technology-based products. At AMI, she is responsible for establishing the value of each innovation and for developing product specific strategic business plans for each one of the new inventions incubated by AMI. In addition, Ms. Gosset manages two AMI programs (cardiology and pediatric solutions). In 2007, she took one of them to full commercialization with nationwide mass market distribution.

Ms. Gosset has a strong engineering background gained from 15 years of engineering leadership. She headed engineering departments for several years and more recently, she specialized in the turnaround of startup companies with special focus on engineering team leadership (VP of Engineering at Sabeus, Director of Engineering at Novera). She worked for large companies (Alcatel and ADC Telecom) and at Alcatel after ten years in optical design engineering (expertise in optical amplifiers and 10 Gbps long distance networks) she became Director of the Program Management Office overseeing the activities of about 600 engineers. She received an Outstanding Achiever Award and five Quality Cups (a prestigious recognition at Alcatel) for innovative leadership.

Ms. Gosset is a member of several medical advisory and director boards and sits on several biomedical review boards for venture firms.

She is the North America representative for the Institute of Electronics and Electrical Engineering - Medicine and Biology Society (IEEE-EMBS), an international organization promoting bioengineering education worldwide.

She has received three prestigious IEEE awards:

- 2007: Career Service award for creating more than 50 chapters worldwide to stimulate innovation between doctors and engineers.

- 2005: IEEE-RAB Leadership award for her contributions to the biomedical community.

- 2005: Outstanding IEEE-EMBS Chapter Award, a global recognition.

Ms. Gosset also supports several charitable organizations where her volunteer work focuses on strategic planning and fundraising strategy. In 2006, she increased the funds to one of her charitable organizations, by a factor of 5 .

Nathalie holds a BSEE from ISEP (Paris, France); MS in Telecommunications (Boulder, CO); MBA (University of St. Thomas, St. Paul, MN).

Ranjan Gupta, M.D. Ph.D.

Dr. Gupta is a tenured Professor and Chair of the Department of Orthopaedic Surgery at the University of California, Irvine. His training began with the Accelerated Biomedical Program with Rensselaer Polytechnic Institute and the Albany Medical College. He completed his orthopaedic surgery residency and NIH post-doctoral fellowship at the University of Pennsylvania. He subsequently completed a hand and microsurgery fellowship at UCLA and an upper extremity/trauma fellowship at the University of Berne, Switzerland. He started as an Assistant Professor at UC- Irvine in 1999 where he established his Peripheral Nerve Research Lab and was accelerated to a full Professor with tenure in 2007. Based on his collaborative work and mentoring of graduate students, he has secondary appointments in the Departments of Biomedical Engineering and Anatomy & Neurobiology. As a surgeon- scientist, he appreciates that the functional recovery after a peripheral nerve injury is often quite limited and focuses his research on Schwann cell control of neural injury. His work has been recognized nationally and internationally with numerous awards including the Marshall Urist Award (Association of Bone and Joint Surgeons), Kappa Delta Award (Orthopaedic Research Society/American Academy of Orthopaedic Surgery), the Chancellor's award for Fostering Undergraduate Research, and most recently, the Sterling Bunnell Fellowship (American Society for Surgery of the Hand).

Kevin E. Healy, Ph.D.

Kevin E. Healy, Ph.D. is a Professor at the University of California at Berkeley in the Departments of Bioengineering and Materials Science and Engineering. He received a Bachelor of Science degree from the University of Rochester in Chemical Engineering in 1983. In 1985 he received a Masters of Science degree in Bioengineering from the University of Pennsylvania, and in 1990 he received a Ph.D. in Bioengineering also from the University of Pennsylvania. He was elected a Fellow of the American Institute of Medical and Biological Engineering in 2001. He has authored or co-authored more than 200 published articles, abstracts, or book chapters which emphasize the relationship between materials and the tissues they contact. His research interests include the design and synthesis of biomimetic materials that actively direct the fate of mammalian cells and facilitate regeneration of damaged tissues and organs. Major discoveries from his laboratory have centered on the control of cell fate and tissue formation in contact with materials that are tunable in both their biological content and mechanical properties. These materials find applications in medicine, dentistry, and biotechnology. He is currently an Assistant Editor of the Journal of Biomedical Materials Research. He has served on numerous panels and grant review study sections for N.I.H. He has given more than 150 invited lectures in the fields of Biomedical Engineering and Biomaterials. He is a named inventor on numerous issued United States and international patents relating to biomaterials, and has founded several companies to develop materials for applications in biotechnology and regenerative medicine.

Andrea Kasko, Ph.D.

Andrea Kasko earned her B.S. in Chemistry in 1997 at the University of Michigan, an M.S.E. in Macromolecular Science at Case Western Reserve University in 1999, and a Ph.D. in Polymer Science 2004 at the University of Akron. She worked as a post-doctoral research associate with the Howard Hughes Medical Institute before joining the Department of Bioengineering at UCLA in September 2006. She is pursuing a research program focusing on the development of novel materials with controllable degradation mechanisms for tissue engineering and drug delivery.

Michael T. Longaker, M.D., M.B.A., FACS

Michael T. Longaker earned his undergraduate degree at Michigan State University, (where he played varsity basketball and was a member of the 1979 NCAA Men's Basketball Championship Team) and his medical degree at Harvard Medical School. He completed his surgical residency at the University of California, San Francisco, a residency in Plastic Surgery at NYU and a craniofacial fellowship at UCLA. The majority of his research training took place while he was a Post Doctoral Research Fellow in the Fetal Treatment Program under Dr. Mike Harrison and in the laboratory of Dr. Michael Banda in Radiobiology, both at UCSF. In December 2003, Dr. Longaker earned his M.B.A. from University of California – Berkeley and Columbia University, in the inaugural class of their combined program. He was elected into Beta Gamma Sigma at Columbia Business School, which is the analogous to Phi Beta Kappa for business programs

Dr. Longaker joined the Stanford University School of Medicine on September 1, 2000 as Director of Children's Surgical Research in the Department of Surgery, Division of Plastic and Reconstructive Surgery and the Lucile Salter Packard Children's Hospital. In 2003, he was named the Deane P. and Louise Mitchell Professor. As Director of Children's Surgical Research, Dr. Longaker has the responsibility to develop a children's surgical research program in the broad areas of developmental biology, epithelial biology and tissue repair, and tissue engineering. Further, Dr. Longaker is the Deputy Director of the Stanford Institute of Stem Cell Biology & Regenerative Medicine, Director of the Program in Regenerative Medicine, Director of Research, Division of Plastic and Reconstructive Surgery, and has been name Professor, by Courtesy, in the Department of Bioengineering. He is also the Faculty Co-Chair for the Stanford University Initiative on Human Health.

Michael Longaker's extensive research experience includes the cellular and molecular biology of extracellular matrix with specific applications to the differences between fetal and post-natal wound healing, the biology of keloids and hypertrophic scars and the cellular and molecular events that surround distraction osteogenesis with respect to craniofacial development. Most recently, his research has focused on multipotent mesenchymal cells derived form adipose tissue and their applications for tissue repair, replacement and regeneration. He brings to Stanford his unique understanding of wound healing, fetal wound healing research, developmental biology and tissue engineering.

Dr. Longaker is a member of all the major academic surgery societies and is currently president of the Society of University Surgeons. He is one of a limited number of surgeons elected into the American Society for Clinical Investigation and was recently elected into the prestigious Institute of Medicine of the National Academies of Science. To date, he has published over 850 publications and has numerous federal grants to support his research.

Jonathan Mansbridge, Ph.D.

Jonathan Mansbridge, PhD, after receiving his degrees from Cambridge University, joined the faculty of the Biochemistry Department of the University of Queensland. On moving to the United States, he took a research post with the Psoriasis Research Institute, investigating keratinocyte differentiation and wound healing. Subsequently, he worked on signal transduction systems in three-dimensional culture at SRI International and on microvascular endothelial cell physiology at Stanford University. He joined Advanced Tissue Sciences in 1993 as Senior Principal Scientist, where Dr. Mansbridge was extensively involved in the research, development and regulatory and commercial aspects of tissue engineering. He was extensively involved in the development, characterization and investigation of TransCyte® for burn wounds and Dermagraft® for diabetic foot ulcers. These projects developed into the general employment of tissue-engineered human extracellular matrix for surgical applications. Currently, he is employed by Smith and Nephew as a Senior Research Fellow, on the development of new processes and products based on three-dimensional fibroblast culture.

Martin Marsala, Ph.D.

Dr. Martin Marsala received his M.D. degree from Safarik University Medical School in Kosice Slovakia in 1986. In 1992 he was awarded a Fogarty International Fellowship to conduct research studies using preclinical models of spinal ischemic injury in the Anesthesiology Research Laboratory at UCSD (sponsor: Dr. Tony Yaksh). During 1994-2000 the major focus of his studies, funded primarily by NIH, included characterization of the mechanisms of induced ischemic tolerance and spinal pharmacology of ischemic spasticity and rigidity. In 2000 he initiated a new series of experiments to characterize a potential therapeutic effect of spinally grafted human neuronal precursors using rodent and minipig models of spinal ischemic injury. At present he is a Professor of Anesthesiology at the University of California, San Diego.

Manuela Martins-Green, Ph.D.

Manuela Martins-Green is Professor of Cell Biology in the Department of Cell Biology and Neuroscience at the University of California Riverside. Professor Martins-Green came to the US from Portugal on a Fulbright Fellowship and received a PhD in Zoology with emphasis in Developmental Biology from the University of California, Davis (1987). She held a postdoctoral fellowship at the Lawrence Berkeley National Laboratory and was Adjunct Assistant Professor at Rockefeller University before joining the UC Riverside faculty in 1993. She is a nationally and internationally recognized researcher in wound healing and tissue engineering and pioneered the role of chemokines in wound healing and angiogenesis. Her research has concentrated on the function of chemokines in wound healing and regeneration, in particular in identifying pathways that lead to scar formation with the goal of reverting them to a regenerative mode. Recently, she has also developed human organ culture systems and engineered mice for studies of wound healing and regeneration. Professor Martins-Green teaches courses in basic Cell and Molecular Biology for graduate and undergraduate students and for graduate students she also teaches in the fields of Wound Healing and Stem Cell Biology. She has served and continues to serve on numerous committees related to scientific programs and education, both in the scientific societies she belongs to and at UCR. In addition to her Fulbright Fellowship for Ph.D. studies in the US, her honors include Postdoctoral National Research Service Award Fellow 1989-1991, UCR Faculty Development Award, 1997-1998; 1999-2000, Department of Defense Breast Cancer Review Panel, Immunology Study Section, 1998-2002, and American Heart Association 2004-2006; Member of the Standing Committee on Women in Cell Biology of the American Society for Cell Biology, 2001-2006. Associate Editor of *Oncology Reports* 1995-1998 and Member of the Editorial Board of "Current Stem Cell Research & Therapy" 2005-present. She has served as member of the Advisory Board for the California Tissue Engineering Meeting since 2003 and more recently of TERMIS. She recently was Chair of the UCR Academic Senate, 2004-2006 and currently serves as Program Co-Chair for the Wound Healing Society Meeting, 2008 and is a member of the committee to hire the new Chancellor at UCR and of the All Campus Committee to develop a UC School of Global Health.

Heather D. Maynard, Ph.D.

Heather D. Maynard received a B.S. in Chemistry with Honors from the University of North Carolina at Chapel Hill in 1992 and a M.S. in Materials Science in 1995 from the University of California, Santa Barbara. Her Ph.D. from the California Institute of Technology was awarded in the summer of 2000 for research in the group of Nobel Prize winner Robert Grubbs. She then moved to the laboratory of Jeffrey Hubbell at the Swiss Federal Institute of Technology (ETH) and University of Zurich, where from 2000-2002 she was an American Cancer Society Postdoctoral Fellow. Dr. Maynard joined the UCLA faculty as an Assistant Professor in August 2002 as the first Howard Reiss Career Development Chair in the Department of Chemistry and Biochemistry and as a member of the California NanoSystems Institute. Maynard has given over 80 invited lectures including the plenary lecture at the UNESCO/IUPAC Conference on Macromolecules in South Africa and the WCC ACS Lecture at Southern Methodist University. Since arriving at UCLA, Maynard has been selected as an Outstanding Emerging Investigator by the Journal of Materials Chemistry and has received the Amgen New Faculty Award, NSF Career Award, Seaborg Award for Outstanding Research in Chemistry, and an Alfred P. Sloan Fellowship.

Anthony Ratcliffe, Ph.D.

Anthony Ratcliffe, Ph.D. is President and CEO of Synthasome, Inc., a biotechnology company in San Diego specializing in tissue engineering and reparative medicine. Dr. Ratcliffe obtained his B.Sc. in biochemistry in 1977, and Ph.D. in immunology in 1980, from the University of Birmingham, UK. He then joined The Kennedy Institute for Rheumatology, London as a Research Scientist, and in 1987 he moved to Columbia University, New York, as Associate Professor of Orthopaedic Biochemistry. In 1996 he joined Advanced Tissue Sciences (then a leading tissue engineering company), where he served as Vice President for Research until 2002, when he founded Synthasome. Dr. Ratcliffe has focused his research on connective tissue biochemistry, musculoskeletal research, and tissue engineering. He has served as a member of the Board of Directors of the Orthopaedic Research Society, Study Sections for NIH, Co-Chairman of the Grant Review Committee for the Orthopaedic Research and Education Foundation, Co-Chairman of the Tissue Engineering Committee for ASTM, and has published more than 100 papers.

A. Hari Reddi, Ph.D.

A. Hari Reddi, Ph.D is the Director of Center for Tissue Regeneration and Repair at the University of California in Davis, California. He is a pioneer in the field of bone morphogenetic proteins and regenerative medicine. Dr. Reddi holds the Lawrence Ellison Chair in Musculoskeletal Molecular biology. He held appointments previously in Johns Hopkins University School of Medicine, the National Institutes of Health and the University of Chicago. He is committed to the mission and goals of the Canadian Arthritis Network.

Susan L. Riley, Ph.D.

Susan L. Riley, Ph.D., is a Senior Manager in the Development Department at Cytori Therapeutics, Inc. She obtained her B.S. degree in Chemical engineering at the University of Minnesota and her Ph.D. from the Chemical Engineering department at Rice University in Houston, Texas under the supervision of Professor Anthony Mikos. Her Ph.D. thesis topic was related to the tissue engineering of bone by culturing marrow-derived stem cells in biodegradable polymer scaffolds. After a brief detour as a postdoc where she investigated how to use ultrasensitive flow cytometry to sequence DNA, she returned to her tissue engineering roots by accepting a Research Scientist position at Advanced Tissue Sciences (ATS). At ATS she studied the effects of scaffold properties on cartilage tissue engineering, as well as being involved in scaling up the production of tissue engineered cartilage constructs. Subsequently she moved to MacroPore Biosurgery as Manager of Polymer Research, being responsible for developing and characterizing new degradable polymeric implants. The Biomaterials division of MacroPore Biosurgery then became a subsidiary of Cytori Therapeutics and she moved into the Biologics Development Department, returning to work with stem cells. Currently, her research focuses on optimizing the isolation process for obtaining stem and regenerative cells from liposuctioned fat tissue.

Robert Sah, Ph.D.

Dr. Robert Sah (ScD, 1990, MIT; MD, 1991, Harvard) joined the UCSDBioengineering faculty in 1992. He was promoted to Professor in 2001, and has served as Vice-Chair since 2002. Dr. Sah's research is on the bioengineering of cartilage and joints in growth, aging, degeneration, and regeneration, with applications to diagnosis, prevention, and treatment of osteoarthritis. His research has been recognized by a Young Investigator Award from the National Science Foundation, a Hulda Irene Duggan Investigator Award from the Arthritis Foundation, and two Kappa Delta Awards from the American Academy of Orthopaedic Surgeons. He is the recipient of the 2006 Van C. Mow Medal from the American Society of Mechanical Engineers. He was named a Professor of the Howard Hughes Medical Institute in 2006. Self-assembled monolayers (SAMs) are widely used to facilitate peptide and protein conjugation to gold surfaces. Alkanethiols with protein reactive moieties produce SAMs with these functional groups exposed on the surface. We have targeted an alkane thiol, HS-(CH₂)₁₁-(OCH₂CH₂)₃-ONH₂, which contains an aminoxy group and a tri(ethylene glycol) spacer for protein resistance. First, the undec-1-en-11-yltri(ethylene glycol) was prepared following literature procedures. Then, N-hydroxyphthalimide was coupled using standard Mitsunobu reaction conditions. The thioacetate group was installed by photochemical addition of thioacetic acid. Removal of both protecting groups resulted in the desired aminoxy alkanethiol. We synthesized SAMs and plan to utilize them for protein conjugation via oxime bond formation.

Michael Siani-Rose

Michael Siani-Rose, President and Founder, has 15 years of biotechnology experience. He has extensive expertise in the computational chemistry and drug discovery fields, and has held managerial and scientific positions at Protos/Chiron Corporation, Kosan Biosciences and Affymetrix. Michael has published 35 peer-reviewed articles, is inventor on seven patents in the field of drug discovery/drug design, and has a dozen patent applications in the fields of bioinformatics and tissue engineering. Michael received his BS degree in Chemical Engineering from the University of Rochester and has done extensive graduate work in Computer Science at University of California, San Diego.

Sharon Stevenson, D.V.M., Ph.D.

Sharon is Co-Founder and Managing Director of Okapi Venture Capital, and is responsible for the Fund's life science investments. Prior to founding Okapi Venture Capital, Sharon most recently served as Senior Vice-President of Technology and Planning for SkinMedica. Prior to joining SkinMedica, Sharon was a Principal with Domain Associates, LLC, a venture capital firm specializing in life sciences investing. In addition to performing due diligence and working closely with portfolio companies, Sharon served as president and chief financial officer of Volcano Corporation, a Domain portfolio company, from 2000-2002, leading the growth of the company from 1 to 14 employees and from the initial design of the first product to the first clinical trials using the device. Her due diligence resulted in initial and follow-on investments of more than \$52 million.

Prior to joining Domain, Sharon was Executive Director of the NeoCyte joint venture between Advanced Tissue Sciences (a San Diego-based biotechnology firm) and Smith & Nephew (a global healthcare firm). Sharon was a tenured Associate Professor in the Department of Orthopedics at the Case Western Reserve University School of Medicine at the time of her departure in 1997. While at CWRU, she was Co-Principal Investigator on grants totaling more than \$4.4 million, authored over 132 publications, and made more than 57 presentations to professional audiences. Her clinical training was in veterinary surgery, and she is Board Certified in veterinary surgery.

Sharon has been awarded an M.B.A. from the UCLA Anderson Graduate School of Management; a Ph.D. in Comparative Pathology from the University of California, Davis; and a Master of Science in Veterinary Pathology and a Doctor of Veterinary Medicine from The Ohio State University.

Bill Tawil, Ph.D.

Dr. Bill Tawil obtained his Bachelor degree in Biochemistry from the University of California at Berkeley. He then completed his Master and Doctorate degrees in Neuroscience at McGill University in Montreal studying the expression and function of adhesion cell surface receptors (integrins) in the Central Nervous System and during tumor metastasis. Subsequently, he spent four years of postdoctoral training at the Center for Cancer Research at MIT under the supervision of Dr. Richard Hynes investigating cell adhesion during the cell cycle. In the last ten years, as a Scientist and a Senior Scientist at U.S. Surgical Corporation and recently at Baxter BioSurgery, he has worked in the field of Tissue Regeneration examining products (synthetic and biologics) that successfully deliver bioactive substances and cells to enhance healing in soft and hard tissue defects. He is presently a director of global strategy at Baxter BioSurgery responsible for scientific initiatives related to tissue engineering establishing collaborations between Baxter and other companies and academic institutions. He is also a director for global marketing for Orthobiologics. Bill is an adjunct professor in the Bioengineering Department at UCLA. Bill also gives lectures about industry and product development in different academic institutions. He is on the Scientific Advisory Committee for various societies including the California Tissue Engineering Meeting and the Los Angeles Tissue Engineering Initiative meeting. Bill believes strongly that the interaction between academia and industry is an expedient and successful way to get products to the patients.

Shyni Varghese, Ph.D.

Dr. Shyni Varghese is currently an Assistant Professor in the Department of Bioengineering at the University of California, San Diego. She joins UCSD following a post-doc at Johns Hopkins University where she worked on osteo-chondro differentiation and three-dimensional tissue formation of adult and embryonic-derived mesenchymal cells using hydrogel-based systems. Varghese's previous postdoctoral research at the University of Notre Dame involved formulating and characterizing novel polymeric materials to be used in orthopedic implants (for spinal fixation and articular cartilage replacement) and tailored for minimal invasive surgery in collaboration with Zimmer Inc. The work involved investigating the role of the reactant chemical structure on photo-polymerization, followed by rheological and mechanical characterization of the resulting networks. Towards her doctoral dissertation, Varghese has experimentally and theoretically investigated the effect of hydrophobic and hydrophilic interactions on biomimicking environmental responsive hydrogels. Her work has also involved understanding novel phenomena in hydrogels, which include volume phase transition, metal complexation, self-organization, and self-healing. Her research at UCSD focuses on developing new strategies using biomaterials and stem cells for tissue repair and regeneration of musculoskeletal tissues. More specifically, she is interested in: (a) developing molecularly-engineered polymeric structures to control the fate and commitment of stem cells towards the desired tissue (b) dissecting cell-environment (insoluble and soluble components) interactions to understand stem cell biology, disease progression, and developmental processes (c) deriving progenitor cells, and (d) translating these fundamental understandings and technologies towards clinical applications in tissue engineering and regenerative medicine.

Ben Wu, D.D.S., Ph.D.

Benjamin Wu, D.D.S., Ph.D., received his D.D.S. from the University of Pacific, his specialty certificate in advanced prosthodontics and complete oral rehabilitation from the Harvard School of Dental Medicine, and his Ph.D. in Materials Science and Engineering from the Massachusetts Institute of Technology. Prof. Wu is currently Vice Chair of the UCLA Department of Bioengineering, with multiple joint appointment in the Department of Materials Science and Engineering, and the Division of Advanced Prosthodontics, Biomaterials, and Hospital Dentistry at UCLA. Benefiting from his unique perspective as a practicing clinician and biomaterials scientist, his research brings to bear bioengineering approach to rebuild lost function in a variety of hard and soft tissues. Several underlying themes that thread his various projects together include 1) learn how nature heals wounds and tissue defects; 2) copy nature and engineer biomimetic microenvironments to promote repair; 3) investigate the mechanisms by which progenitor cells interact with the engineered microenvironments; and 4) investigate the mechanism by which biomolecules interact with various scaffolding materials. The multidisciplinary nature of his research is reflected by his role as Co-Director of the Weintraub Center for Reconstructive Biotechnology, and his membership in the Brain Research Institute, the California NanoSystems Institute, and Cardiovascular Stem Cell Research Center at UCLA.



ABSTRACTS

Session I: Biomaterials Engineering

Session II: The California Triple Helix in Regenerative Medicine

Session III: Stem Cell Biology & Tissue Engineering

Session IV: Cytokines and Cell Signaling in Tissue Engineering

SYNTHESIS AND CHARACTERIZATION OF ENZYMATICALLY CROSSLINKED PROTEIN POLYMER HYDROGELS FOR TISSUE ENGINEERING APPLICATIONS

Annelise E. Barron

Stanford University, Department of Bioengineering

We have utilized genetic engineering and bacterial expression to create high-molecular weight protein polymers that can be enzymatically crosslinked into viscoelastic hydrogels suitable for tissue engineering applications. The synthesis of protein polymers by biological methods allows for precisely controlled protein length (monodispersity) and a specifically tailored amino acid sequence (controlled reactivity). Controlled cloning* and recombinant protein technology were used to produce highly repetitive protein polymers $(GKGSGKGA)_x$, $(GKGTGA)_n$, and $(GKAGTGSA)_m$ with lengths $x = 15, 30$, $n = 20, 40$ and $m = 30, 60, 120$ respectively. Subsequently, a block copolymer was created with the sequence $[(GQ3LG2AGTGSA)_2(GAGQGSA)_3]_6$. In solution, these proteins are unstructured (random-coil), and are water-soluble up to 75°C as confirmed by circular dichroism spectroscopy and turbidity experiments. These lysine-containing protein polymers are also susceptible to protease digestion. For example, the serine protease, plasmin, as well as trypsin effectively cleaved soluble proteins into smaller fragments on a short time scale.

We have examined the physical properties of hydrogels formed by crosslinking these protein polymers in various ways. After chemical crosslinking with different amine-reactive crosslinkers, we found that the degree of hydrogel swelling is strongly affected by the crosslink density and buffer conditions. Depending on crosslinking conditions, hydrogels can be formed with storage moduli (G') ranging from 100 to almost 100,000 Pa. Current experiments are focusing on enzymatic gelation using transglutaminase (TG). TG forms an acyl bond between the ϵ -amino group in our lysine-based proteins and the glutamine residues in the second group of protein polymers. Currently, we can form enzymatic gels in less than 2 minutes. In addition, we have begun to characterize the hydrogels viscoelastic, degradation, and swelling properties. We believe that we can form a class of in situ gelling materials that have a range of properties that can be tailored to meet specific biomedical application requirements.

* J.I. Won, A.E. Barron, *Macromolecules* 2002, 35, 8281.

DESIGNING A MATRIX TO RECREATE THE OPTIMAL MICROENVIRONMENT FOR A TISSUE ENGINEERED CORNEA

Davidson, MSS, Orwin, EJ Dept. of Engineering, Harvey Mudd College, Claremont, CA

INTRODUCTION: The overall goal of our project is to create a tissue-engineered corneal replacement. In the native cornea, corneal keratocytes thrive in a three-dimensional scaffold of 30 nm collagen fibers with small amounts of glycosaminoglycans (GAGs) such as chondroitin sulfate (CS). Along with collagen, the GAGs form proteoglycans, such as biglycan, decorin, and lumican, which help regulate fiber spacing and stability of the ECM of the cornea. Natural collagen fibers in the cornea exhibit the well-documented banding pattern perpendicular to the direction of the fiber with a banding period of 67 nm. Thus far attempts in our lab to recreate the microstructure of the corneal stroma have only included a collagen component. In addition, previous work in the lab has shown that cell media can dissolve collagen scaffolds, making cell culture impossible. In this project, we investigate methods for optimizing the production of a scaffold for corneal tissue engineering which is cell culture-ready. The objectives of this project are (1) to incorporate chondroitin sulfate into the electrospun collagen scaffolds to better mimic the microenvironment of the cornea, (2) to optimize the crosslinking method to ready the scaffold for cell culture, and (3) to observe the banding pattern in electrospun collagen to verify that these fibers provide a natural environment for cells.

METHODS: Collagen fibers and collagen-CS fibers were formed through electrospinning. Electrospinning was performed by applying a high potential (roughly 10 kV) across the gap between the grounded collection plate and a syringe containing the collagen in amino acid solution. Crosslinking was performed with combinations of the following methods. Glutaraldehyde vapor treatment was performed by allowing samples to sit in a dessicator with a 25% glutaraldehyde solution. Dehydrothermal treatment was performed in a vacuum oven at 120C and 36 torr. All samples also underwent treatment with 0.1% liquid glutaraldehyde for one hour. PureCol (control type I collagen fibers) and electrospun collagen fibers were examined using a Nanoscope IIIa Atomic Force Microscope using a silicon nitride tip with a spring constant of 40 N/m.

RESULTS: Certain concentrations of chondroitin sulfate in collagen formed satisfactory fibers during electrospinning, and some did not. Preliminary results show that the newly developed method adequately crosslinks collagen fibers. The banding pattern was observed in PureCol collagen fibers. Although it was not observed in electrospun fibers, samples were much too thick to obtain high vertical resolution.

DISCUSSION: Matrices produced with chondroitin sulfate and collagen could provide a more appropriate microenvironment for corneal keratocytes with the potential to convey appropriate signals to maintain the proper cell phenotype in culture. In addition, we developed a new crosslinking protocol which requires three days to complete, a significant improvement over the previously required four days. We were able to resolve the banding pattern in positive control collagen fibers with our AFM images, but are still working on producing thin enough electrospun samples to provide adequate resolution to image the fibers.

MAGNETICALLY ACTUABLE SCAFFOLDS FOR TISSUE REGENERATION

Julia J. Mack,¹ Abigail A. Corrin,¹ Brian N. Cox,¹ Min Lee,² Mandy Lam,² James C.Y. Dunn² and Benjamin W. Wu²¹Teledyne Scientific, Thousand Oaks, California, USA²Department of Bioengineering, UCLA, Los Angeles, California, USA

INTRODUCTION: Achieving structure and shape in an aggregate of cells is still a challenge in tissue engineering. Control of cell growth and differentiation could be improved with the addition of two mechanical functionalities to existing scaffolds: the ability to pump fluids and the ability to impose mechanical strains on the cells during growth. These functions could be effected in a non-intrusive manner if scaffolds are developed that can be actuated by the remote application of magnetic fields. Presented here are methods to fabricate magnetically modified biocompatible polymer scaffolds, which can be actuated by remotely applied magnetic fields. The magnitude of the actuation is shown to be biologically useful by simple tests in known magnetic fields and magnetic field gradients. Methods of processing the functionalized polymers into 3-D scaffolds have been demonstrated, suggesting wide applicability in tissue engineering.

METHODS: Dispersions of magnetic nanoparticles (α -Fe₂O₃) in a biodegradable polymer matrix (PLGA or PCL) were prepared with concentrations ranging from 5 to 30 weight percent α -Fe₂O₃ (based on polymer). Composite fibers were formed via electrostatic spinning. A tube is formed by rolling the fibril layer around a mandrel of desired diameter until the desired tube wall thickness is achieved. The number of turns to achieve a given thickness can be varied by electrospinning fibril layers of varying thickness. This can be done over the range of interest (fibril layer thickness 5 – 200 μ m). To form a 3-D scaffold populated with cells, the electrospun fibril sheet is seeded with cells and subsequently wound into a tube structure.

RESULTS: The application of a magnetic field to a composite of magnetic nanoparticles (α -Fe₂O₃) dispersed in a non-magnetic medium results in body forces and moments distributed throughout the material. Actuable scaffolds have been formed by electrospinning sheets of magnetically functionalized polymer fibrils. Smooth muscle cells are then seeded on the sheets of electrospun composite fibrils. Winding of the cellseeded fibril sheets results in magnetically actuable 3-D scaffolds. We have chosen a winding method of creating the tube because it allows the build-up of walls that are much thicker than a single layer without joining separate segments. The tube has the gross morphology of intestine or urethra.

DISCUSSION: Our objective is to develop actuable scaffold materials that move under the action of a magnetic field, which can be applied remotely. Thus, an implanted scaffold will be actuated in vivo by a non-invasive action. Controlled variation of the field is achieved by moving the permanent magnet relative to the actuable scaffold. To ensure consistent field variations, we have built a robotic arm, which holds and moves the magnet according to instructions from a controlling computer. The fibrillar scaffolds are shaped as tubes, set up so that an approximation of peristaltic motion can be generated by magnetic actuation.

BIOSURFACE ENGINEERING

Karen L. Christman, PhDUCSD, Department of Bioengineering

Over the past few decades, techniques to produce submicron and nanoscale features on surfaces have emerged. While such advances were initially applied to the electronics field, the fusion of biology and nanotechnology has begun to provide useful tools for biomaterials and tissue engineering applications. The ability to spatially orient and anchor proteins in particular affords many opportunities for biotechnology and medicine. Site-specifically immobilizing proteins, forming protein assemblies, and fabricating three-dimensional biological nanostructures using various polymers and lithography techniques will be discussed.

ELECTROSPUN POLYCAPROLACTONE NANOFIBROUS SCAFFOLD FOR LIVER TISSUE ENGINEERING

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INTRODUCTION: Tissue engineered structures are fast becoming the paradigm of choice for the repair and replacement of diseased and injured liver tissue. One of the attractive resorbable polymers for the preparation of porous scaffolds is polycaprolactone because it can be electrospun into nanofibrous webs. The objective of this study was to determine the optimal electrospinning conditions for preparing scaffolds that would support the culture and cellular growth of hepatocytes.[1,2]

METHODS: Polycaprolactone (PCL) (Mw 65,000) (Sigma-Aldrich) with a melting point of 60°C and a density of 1.145 g/cm³, was dissolved in a 3:1 ratio of chloroform and methanol solvents. A spinning solution made by dissolving 10% by weight of PCL was used for all the electrospinning trials, which employed the use of a vertical parallel plate electrospinning system. The top plate was grounded, and the bottom plate was attached to the positive power supply of 48 kV, hence serving as the collection plate for the spun nanofiber web. A meltblown polypropylene nonwoven fabric served as the collection medium. After sterilization in UV light, each sample was soaked in culture media for 2 hours before cell seeding. The standard hepatocyte American Type Culture Collection cell line AML12 (ATCC Catalog No. CRL-2254) was used following a static culture technique [3]. The presence and density of cells attached to the nanofibrous scaffolds were observed using scanning electron microscopy. For a live/dead cell viability test the fluorescence cytotoxicity assay for mammalian cells was used.

RESULTS: After 7 days in culture, the initial tests showed a strong growth of hepatocytes. In terms of the number of live hepatocytes present, the fluorescence assay showed similar density and morphology for both the PCL nanofibrous scaffolds and cellular control sample. More cell growth was seen after 14 days on the PCL nanofibrous scaffolds. The material control of just polypropylene showed significantly less growth than those with the PCL nanofibers present. SEM images confirm that the cells are not only attached to the PCL nanofibrous scaffolds, but are confluent and well-adhered. ELISA tests were run using albumin as a marker.

DISCUSSION: The initial results showed positive cell growth on the PCL nanofibrous scaffolds. The hepatocytes were found to proliferate similarly to those cultured on the control surfaces without nanofibers. SEM analysis shows the adhesion of the confluent cells to the scaffold and the ability of the nanofibers to direct the proliferation of the cells. In fact, the ELISA tests show there is an increased functionality of the hepatocytes at both 7 and 14 days with the PCL nanofibrous scaffolds than with no scaffold present. The scaffolds will be used to encourage hepatocyte grow on a substrate that can be used in tissue engineering and organ regeneration. Further studies will be undertaken to determine the viability of liver cells at longer time points. Concurrently, the use of a dynamic culture technique will also be investigated.

REFERENCES:

- [1] Sakai Y, Mater. Sci Eng C, 2004;C24: 379-386
- [2] Reneker DH, Polymer, 2002;43:6785-6794
- [3] Yasuda, K. Tissue Eng. 2004; 10:1587-1596

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TRANSFERRING NOVEL TISSUE ENGINEERING AND REGENERATIVE MEDICINE DISCOVERIES FROM THE UC TO THE PUBLIC

Kathryn A. Atchison, DDS, MPH Vice Provost, Intellectual Property and Industry Relations

Many important discoveries such as biomaterial matrices, scaffolds of fibers derived from a biodegradable polymers and other tissue engineering applications begin in the laboratories at UCLA. These technologies offer the potential to create new options for the public. UCLA strives to be in the forefront on technology commercialization through practices for innovative partnering with industry. The goal for intellectual property management at UCLA is to protect university's status as a pre-eminent education and research university while facilitating collaboration between faculty and industry for rapid R&D and technology commercialization. Examples of best practices will be presented that strive to offer industry a corporate alliance team that philosophically and operationally integrates the management of industry sponsored research, material transfer, and licensing agreements into a single portal for company sponsors..

THE TISSUE ENGINEERING DIVIDE: AN EXPLOSION IN DISCOVERY, AN INDUSTRY IN PERIL

Dawn R. Applegate, Ph.D. President & CEO, RegeneMed, Inc.

The estimated \$100B organ replacement therapy market has proven elusive. Maximum annual sales of any one tissue engineering, gene therapy or cell-based therapy product is approximately \$65M. While companies such as Organogenesis and Advanced Tissue Sciences invested approximately one-tenth of what pharmaceutical companies expend on drugs (\$1B/drug) to bring these tissue engineering products to market, sales of the tissue engineered products have been an abysmal one-thousandth that of a blockbuster drug. The economics do not support the risk of the investment. Consequently, venture capital is scarce. Despite large and rapidly increasing funding from the NIH and other government sources, roadblocks in clinical trials and FDA approvals coupled with disappointing sales have pushed two leading tissue engineering companies along with a disconcerting following of others into bankruptcy and has halved the industry's market valuation.

We are facing a cart-and-the-horse dilemma: a post-dot-com-crash renewed interest in funding will be required to launch a blockbuster cell-based product which can realistically only occur in today's conservative market upon the success of such a product. As a result, most of the funding in the biotechnology space is going to proven and quick to market tracks such as monoclonal antibodies, small molecules and medical devices, preferably 510K medical devices. The result is a growing divide between academia and industry in research on cell-based therapies. Students trained in the field are increasingly frustrated as they are forced to negotiate a fit for their expertise to companies developing small molecules, biologics and medical devices.

Given these conditions, researchers in the field should consider technology development for both near and far-term applications, including non-therapeutic applications. There is much to be gained from industry familiarity and experience with technology, even if the application is not the researcher's intended use or for the largest potential market. Moreover, to market a product or even enter clinical trials, a great deal of data is required on mundane product attributes, such as stability, shipping, ease of use and aesthetics. Obtaining this information on a less expensive, less regulated product application will reduce cost and facilitate technology advancement to critical care applications.

Examples of successful technology transfers and leveraging novel concepts into short-term revenues aimed at fueling long-term therapeutic applications will be presented. Pitfalls and triumphs will be highlighted. Methods by which researchers can effectively interact with industry and become entrepreneurial themselves will be emphasized with the goals of developing best practices for accelerating the advancement of biomaterial and cell-based medical technologies.

ADVANCING THEREGEN'S CELL-BASED EPICARDIAL PATCH THROUGH THE EVOLVING REGULATORY AND PUBLIC POLICY ENVIRONMENT

Michael Siani-Rose, Theregen, Inc. 225 Bush Street, Floor 16,
San Francisco, CA 94104

Technology

The purpose of Theregen's current Phase I human study entitled "A Phase I Open Label Pilot Study to Evaluate the Safety, Efficacy and Tolerability of Anginera™ for Adults With Left Ventricular Dysfunction AND Reversible Myocardial Ischemia Undergoing CABG Surgery" is to evaluate the safety and biological effect of using a bioresorbable scaffold-based, three-dimensional, human dermal fibroblast culture (Anginera™) to treat diffuse small vessel disease in human hearts. It has been hypothesized that treatment with Anginera, in conjunction with a Coronary Artery Bypass Graft (CABG), would be safe and improve ventricular performance and wall motion in these hearts after treatment.

Regulatory Issues

Theregen's approach to repurposing a cell-based patch for cardiac applications has presented several hurdles, overcome by aspects unique to Theregen's business model: Acquired rights to a cell-based product (previously-approved for diabetic foot ulcers) for a new cardiac indication; Used human data from a different application (diabetic foot ulcers) and previously-developed animal model studies to advance into humans; Navigated the regulatory pathways in moving the product from Device (CDR) to Biologic (CBER); Developed an indication that allows patch placement on the heart in conjunction with another therapy (e.g., CABG) to demonstrate safety and evidence of new perfusion and increased heart function; Worked with the FDA to address concerns about application of a cell-based therapy placed directly on the surface of the heart

Recommendations

The accelerating pace of regenerative medical research and development requires a corresponding increase in substantive communications from all affected sectors. Increased interchange between companies and the academic community for developing research and clinical models that address the evolving regulatory environment; Forums/workshops to discuss the blurring of regulatory lines between pharma, biotech and cell-based therapies; Leadership from industry associations in partnership with the California Institute for Regenerative Medicine and academia.

USING BIOMATERIALS IN DIFFERENTIATION STRATEGIES WITH HUMAN EMBRYONIC STEM CELLS

Amander Clark, Ph.D. UCLA Stem Cell Institute

Human Embryonic Stem Cells (hESCs) hold remarkable promise for regenerative medicine, and as a tool for understanding human embryo development. Prior to the use of hESCs, identifying molecules and signaling pathways that regulate differentiation of the early human embryo was impossible due to ethical and moral constraints. My laboratory studies derivation and function of human germ cells (eggs and sperm). Germ cell formation is initiated from the pluripotent cells of the peri-implantation embryo therefore hESCs constitute the only genetically malleable cell-based model to study their genesis and development. Understanding human germ cell formation is important because abnormal differentiation results in infertility, germ cell tumors and if fertilized-fetal and infant morbidity, mortality and birth defects. My laboratory uses three independently derived lines of hESCs as models to study human germ cell differentiation. We have found that all three lines of hESCs form germ cells under spontaneous differentiation conditions. By incorporating various biomaterials into our differentiation strategies, we can recover distinct developmental stages of human germ cell development from the three independently derived hESC lines. Therefore, our results indicate that specific paracrine signals differentially expressed between lines of hESCs are essential for enabling germ cell formation, and that use of chemically defined biomaterials will be essential to dissecting the identity of these signals.

MESENCHYMAL STEM CELLS DIFFERENTIATION INTO OSTEOBLASTS ON 2D AND IN 3D FIBRIN SCAFFOLDS

Melissa Chow, Haison Duong, Benjamin Wu, Bill Tawil
UCLA Department of Bioengineering

INTRODUCTION: Mesenchymal stem cells (MSCs) have the potential to differentiate into a wide variety of cell lineages, including bone. Fibrin, which is biocompatible and bioresorbable, has several advantages over other types of biomaterials that are currently being used in tissue engineering. In this study, we examined how different biomaterials affect MSCs proliferation and osteogenic differentiation on 2-D monolayer cultures and in 3-D fibrin scaffolds of varying fibrin concentrations.

METHODS. 2-D monolayer cultures were created by seeding hMSCs, fibroblasts, and osteoblasts in 24-well plates pre-coated with various substrate materials: fibrinogen, collagen, fibronectin, or un-coated polystyrene. Cells were cultured in either normal growth medium or in osteogenic inducing medium. At various time periods, cultures were assessed for osteogenic indicators using alkaline phosphatase (ALP) and Von Kossa staining. In parallel, cells were embedded in 3D fibrin scaffolds and cultured for same period of time. Cell proliferation was then determined by Alamar Blue dye reduction assay and mechanical stiffness was determined by tensile measurement by an Instron machine.

RESULTS: In the 2-D study, cells exhibited more ALP positive staining in presence of an inducing osteogenic medium, most notably, hMSCs that were seeded atop the fibrinogen substrate. VonKossa staining showed that in osteogenic medium, the hMSCs on collagen produced the most calcium deposits. Results from the 3-D fibrin scaffold model showed that hMSC proliferation is inversely related to fibrinogen concentration. Formulations containing lower fibrinogen concentrations resulted in higher hMSC proliferation, confirming previous studies [1,2]. Cell proliferation was greater when cells were cultured in osteogenic medium. The mechanical testing results demonstrate that hMSC-populated fibrin gel formulations with higher fibrinogen concentrations have a greater mechanical stiffness.

DISCUSSION: We have shown that hMSC proliferation and differentiation is dependent on type of substrates they are seeded on, most notably, fibrinogen; thereby, embedding cells in scaffolds using this component may create an ideal environment for cell growth and differentiation. Knowing which formulation of fibrin components is best suited for cell growth will allow us to manipulate hMSCs differentiation toward particular cell types i.e osteoblasts. Future studies will involve inducing hMSCs to differentiate into osteoblasts and generating bone matrix materials.

REFERENCES:

- [1] Catelas, I., Nadjah, S., Wu, B., Dunn, J., Helgerson, S., Tawil, B. "Human Mesenchymal Stem Cell Proliferation and Osteogenic Differentiation in Fibrin Gels in vitro." *Tissue Engineering* 2006; 8: 2385-2396.
- [2] Ho, W., Tawil, B., Dunn, J., Wu, B. "The Behavior of Human Mesenchymal Stem Cells in 3-D Fibrin Clots: Dependence on Fibrinogen Concentration and Clot Structure." *Tissue Engineering* 2006; 12: 1587-1595.

IN VIVO CARTILAGE REGENERATION USING HUMAN EMBRYONIC STEM CELL-DERIVED MSCS

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INTRODUCTION: Significant advances have been made in recent years using stem cells as a cell source for cartilage tissue regeneration and repair. Human embryonic stem cells (hESCs) have the potential to self-renew and differentiate into a wide range of lineages including the chondrogenic lineage. The precise microenvironments required for differentiation of hESCs are only beginning to emerge and the hESC based in vivo tissue engineering remains a relatively unexplored field. Here we describe the generation of functional cartilage tissue in vivo from hESC-derived mesenchymal cells by providing a microenvironment comprising of chondrocyte secreted morphogenetic factors and 3-dimensional (3D) poly (ethylene glycol)-(PEG) based hydrogel support. Treatment of chondrocyte-conditioned medium was sufficient to induce the lineage commitment and long-term survival of hESCs in vivo. In addition, the cells transplanted into the cartilage lesion repaired the defects by differentiating into chondrocyte and forming mechanically stable articular cartilage.

METHODS: hESC line (Hues9) was cultured as previously reported¹. Mesenchymal cells were derived as described previously². For conditioned medium (C-MSCM), DMEM was incubated with primary chondrocytes for 48 hrs, passed through a 0.22 mm filter, and supplemented with 10% FBS, 2mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. For hydrogel encapsulation, polymer solution was prepared by mixing 10% (w/v) PEGDA in sterile PBS. Cells were gently mixed and polymerized with 365 nm light (4.5 mW/cm²) at a concentration of 2×10⁷ cells/ml. The constructs were then implanted subcutaneously into the dorsal region of 6 to 8-weeks-old athymic mice for duration of 12 weeks or transplanted into knee defect (~2 mm defect) of athymic nude rat and sealed with fibrin glue (n=6).

RESULTS: Upon stimulation with chondrocyte-secreted factors, hESC-derived mesenchymal cells upregulated cartilage-specific markers such as Sox-9, aggrecan, type II collagen, and link protein both in vitro and in vivo conditions. Conditioned medium expanded cells displayed type II collagen, indicating chondrocyte phenotype. In addition, in vivo engineered cartilage with these cells exhibited greater biochemical contents with enhanced mechanical properties. Ultrastructure of encapsulated cells, examined by electron microscopy, displayed cell morphology characteristic of chondrocytes and these cells were surrounded by an abundant ECM. Most of the morphogenetic factor-induced cells maintained survival after 12 weeks. These results confirm that the morphogenetic factors from chondrocytes induced long term in vivo commitment into chondrocytic phenotype and promoted survival of cells after transplantation.

DISCUSSION: Successful use of hESC-derived cells for clinical application in cartilage regenerative medicine requires tight control of the cell differentiation process resulting in relatively pure population of therapeutically relevant chondrocytic cell types. The present study is the first to demonstrate the feasibility of tissue engineering of hyaline cartilage from mesenchymal cells derived from hESCs.

ACKNOWLEDGEMENTS: This work was supported by funding from the Johns Hopkins University (JHU)-Technion Joint Program and the Whitaker Foundation.

REFERENCES: 1. Cowan, C. A. et al. *N Engl J Med* 350, 1353-6 (2004). 2. Hwang, N. S. et al. *Tissue Eng* 12, 2695-706 (2006).

SPINAL ISCHEMIC PARAPLEGIA: MODULATION BY STEM CELL IMPLANT

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Transient spinal cord ischemia represents a serious complication associated with aortic cross-clamping i.e. a procedure required in order to repair an aortic aneurysm. Depending on the duration of the ischemia the resulting neurological deficit can clinically be expressed as flaccid or spastic paraplegia often combined with rigidity. Histopathological analysis of spinal cord segments in animals suffering from spasticity and rigidity show a selective loss of small inhibitory GABA-ergic and glycinergic interneurons primarily in the intermediate zone in lumbosacral segments but with continuing presence of ventral α -motoneurons. Consistent with the loss of segmental inhibition, electrophysiological analysis in animals with chronic spasticity and rigidity show, i) an increased amplitude of spontaneous and cortically evoked potentials recorded from peripheral muscle, ii) an increased peripheral muscle tone as quantitatively measured by resistance to ankle flexion and corresponding increase in EMG activity, and, iii) significant increase in Hoffmann reflex. In recent studies we demonstrated that spinal grafting of rat or human spinal neuronal precursors or postmitotic human hNT neurons in rats with ischemic spasticity is associated with a significant qualitative and quantitative improvements in ambulatory function, reduced motor evoked potentials and suppression of otherwise increased muscle tone. This recovery of function corresponded with a robust maturation of grafted neurons, extensive axodendritic sprouting of identified implanted cells and development of inhibitory (GABA/glycine) and excitatory (glutamate) synapses between grafted cells and persisting host α -motoneurons. More recently we have demonstrated a comparable graft survival, neuronal differentiation and synapse formation in nude rats or immunosuppressed minipig. These data showing behaviorally relevant changes in spinal function consistent with developing synaptic contacts suggest that a region specific grafting of neuronal precursor or fully postmitotic neurons will have therapeutic utility in patients suffering from spinal ischemia-induced spastic paraplegia.

CHARACTERIZATION AND TRANSPLANTATION OF NEURAL CREST PROGENITOR CELLS

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INTRODUCTION: Hirschsprung's disease is a congenital megacolon due to the lack of a functional enteric nervous system. Patients suffering from this have a function obstruction and usually require surgical intervention. Multipotent neuronal progenitor cells have been isolated and may have the potential to repopulate this aganglionic region of the gut. Low affinity nerve growth factor, p75, has been reported as a maker for neural crest progenitor cells that may be capable of regenerating the enteric nervous system.

METHODS: Primary harvest cells were obtained from intestinal smooth muscle strips from P2-P3 Lewis and Lewis-GFP transgenic rat pups. For in vitro experimtns, cells were cultured in DMEM-low supplemented with chicken embryo extract, B27, N2 bFGF, IGF, retinoic acid and α -mercaptoethanol (Morrison 1999). Cells were also sorted with FACS to select cells that express the highest level of p75 (~7-8%). For in vivo experiments, cells were seeded onto an electrospun PCL tubular scaffold or directly injected with a 33G needle. Cells or animals were sacrificed at various time points for analysis.

RESULTS: In vitro cultures of p75-high expressing cells have shown a significant growth potential over the control populations of cells. In vitro cultures have also shown the expression of p75, S100, peripherin and α -smooth muscle actin at various times points. In vivo experiments have shown peripherin and S100 staining in transplanted scaffolds seeded with cells and cells that were directly injected.

DISCUSSION: Cells expressing high levels of p75 have shown great growth potential, express various markers and survive transplantation in vivo. While further studies are required, the cells continue to show great potential for regenerating the enteric nervous system.

REFERENCES:

1. Morrison, S. J., Prospective identification, isolation by flow cytometry, and in vivo self-renewal of multipotent mammalian neural crest stem cells. Cell 96, (1999)

USING TISSUE ENGINEERING TO STUDY CYTOKINE FUNCTION Manuela Martins-Green, Department of Cell Biology and Neuroscience, University of California Riverside, CA 92521

One of the goals of tissue engineering is to rebuild lost body parts. For many years, transplantation of parts from one individual to another was used to overcome these problems. Although successful in many regards, transplantation faces 2 major problems, one being immune rejection and the other unpredictable availability. It was within this framework that Tissue Engineering was born. In essence, we seek to construct tissues in which living cells and matrices are brought together along with a variety of survival factors in specific structural contexts that can be used to alleviate missing tissues and parts. Another objective of tissue engineering is to create these organ cultures to provide an environment that mimics human tissues to study cell function under a variety of conditions and hence unravel normal and disease processes without having to experiment on humans directly. It is within this context that I wish to present this talk. We have developed several complex systems in culture that have allowed us to probe fundamental biological questions of human biology. In this presentation I will show data using a human-like skin to study angiogenesis, a model system to study increases in permeability of microvessels during inflammation, a human-like arterial wall to study very early stages of atherosclerotic plaque development, and a mouse model system in which we have knocked in a gene to study cytokine function. Detailed presentation of all of these systems and the research will also be done in poster format.

IN VITRO MODELS TO STUDY MYOFIBROBLAST ACTIVATION

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INTRODUCTION: Fibrosis is the accumulation of excess extracellular matrix, a chronic proliferative scar. Chronic fibrotic disease is a causative factor in conditions such as end stage renal failure and idiopathic pulmonary fibrosis (IPF), and is estimated to play a role in 45% of deaths in the developed world. There are currently no effective therapies. Connective tissue is created by fibroblasts that synthesize the matrix and remain relatively quiescent in normal adult tissues. During wound healing fibroblasts become activated to become myofibroblasts that replace the lost connective tissue and provide the tensile forces required to contract the wound. The causes of most fibrotic conditions are not known, but fibrotic tissues are characterized by the presence of myofibroblasts, and fibrosis is often thought of as an aberrant wound healing process. CCN2 (Connective tissue growth factor, CTGF) is a small, secreted protein that is highly expressed during wound healing and in fibrotic conditions. In all fibrotic diseases studies the level of CCN2 expression correlates well with the severity of disease. Inhibition of CCN2 in animal models results in decreased fibrosis and thus CCN2 has emerged as an attractive drug target.

METHODS: We are examining the role of CCN2 in the activation of fibroblasts to myofibroblasts using isolated primary dermal fibroblasts in 2-D culture, 3-D lattices of collagen type I, and in polyethylene glycol (PEG) based hydrogels.

RESULTS: Myofibroblasts express a smooth muscle actin (aSMA) that causes them to be contractile, and the form large focal adhesions, focal points where the cell localizes molecules involved in cell adhesion and migration. Dermal fibroblasts derived from Ccn2 null mice are deficient in collagen gel contraction, a measure of fibroblast activation, suggesting a role for CCN2 in the conversion to activated myofibroblasts. However, we have found that the Ccn2 null fibroblasts express aSMA to the same extent as the WT cells, and the null cells also form the larger 'supermature' focal adhesions that are typical of myofibroblasts. Fibroblasts adhere to PEG hydrogels that include a small peptide sequence, RGD, that allows cells to adhere through a subset of integrins, cell surface receptors for the extracellular matrix. We have found that Ccn2 null cells can adhere to PEG-RGD gels, although these cells express less integrin $\alpha 5$ that mediates RGD binding. Fibroblasts only form focal adhesions on the more rigid gels, and there is no obvious difference between WT and Ccn2 null cells. We are using degradable lattices to create mechanical tension in the system after the cells have adhered to examine how this will affect the morphology of the cells, formation of focal adhesions and conversion to the myofibroblast phenotype.

DISCUSSION: These data suggest that CCN2 may not be directly involved in the initial conversion to myofibroblasts, but instead may have a role in the persistence of myofibroblasts in fibrotic conditions. Our ultimate goal with these studies is to develop defined PEG based hydrogels to study the conversion of fibroblasts to myofibroblasts in more physiologically relevant 3-D environments than are currently achievable with traditional culture systems.

MECHANOTRANSDUCTION OF CHRONIC NEURAL INJURY VIA SCHWANN CELLS

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One of the central paradigms of neuroscience is that the peripheral nervous system may regenerate while the central nervous system does not. As most clinicians appreciate, functional recovery after a peripheral nerve injury is most often quite limited. The fundamental question that shapes our research program is how a sustained mechanical stimulus to a peripheral nerve is transduced into a biochemical response- i.e., to understand the molecular pathogenesis of compression neuropathies such as carpal tunnel syndrome, cubital tunnel syndrome, and spinal nerve root stenosis. Until recently, they were simply considered variants of acute nerve injuries that simply developed over time and had a similar pathogenesis. Much of the earlier literature described compression neuropathies as the by-product of mild Wallerian degeneration that occurred at the site of injury. Recent research using both in vivo and in vitro modeling systems has called that notion into question. The hallmark of compression neuropathies is a progressive decrease in the nerve conduction velocity at the site of injury that occurs over time. Yet, there are numerous changes at the site of injury that actually occur before the nerve conduction velocity changes. Among them, one of the primary features is a dramatic Schwann cell turnover that induces both Schwann cell proliferation and apoptosis in the absence of any evidence of axonal pathology. This finding is quite remarkable as glial cells such as Schwann cells most often respond secondarily to the cues from neurons, as with acute nerve injuries and Wallerian degeneration. Moreover, there is a progressive change in the Schwann cell phenotype as these cells become less promyelinating, with a decrease in myelin-specific proteins such as myelin basic protein and myelin-associated glycoprotein (MAG). In turn, they up-regulate pro-regenerative molecules such as vascular endothelial growth factor. This appreciation of the central role of Schwann cells in the pathogenesis of compression neuropathies will help improve management of these conditions with the potential of developing effective treatment regimens to reverse this pathology.

THE SPATIAL DYNAMICS OF EPITHELIAL POPULATION GROWTH

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INTRODUCTION: A key to success in tissue engineering is to provide essential microenvironmental cues that allow cells to repair damaged or lost tissue. An example is the delivery of soluble growth factors such as epidermal growth factor (EGF) to an injured skin site for burn or ulcer treatment. A major requirement in these applications is to control the spatial and temporal availability of these factors to achieve rapid and efficient tissue ingrowth without releasing excessive amounts of growth factors. This challenge is especially important in epidermal tissue regeneration where complete surface coverage is required for the integrity of the regenerated tissue. Therefore, to precisely manipulate epithelial proliferation in a desirable manner, it is essential to have a quantitative and mechanistic understanding of epithelial growth dynamics driven by soluble growth factors.

METHODS: MCF-10A (a non-tumorigenic mammary epithelial cell line) cells were exposed to different concentrations of EGF. Cell growth and EGF concentration profiles were observed overtime. By using immunofluorescence against BrdU, diphospho-ERK, and phosphor-Akt, we assayed DNA synthesis and EGF-mediated intracellular signals among peripheral and central cells for each condition.

RESULTS: One of the most distinct phenotypes of epithelial cell cultures is that cells are grouped into clusters. Our data reveal significant spatial gradients in growth dynamics within these epithelial cell clusters. At early time, both central and peripheral cells proliferate equally well. However, as cell clusters mature, cells at the center fail to divide, while peripheral cells continue to proliferate. Ultimately, all cells become quiescent, and population growth ceases. These observations reveal that population growth occurs in three phases. We discovered that the onset of different phases of population growth correlate with the extent of growth factor depletion by receptor-ligand trafficking. Furthermore, we found that central cells undergo a process of compaction and ERK signals are preferentially attenuated among them during the intermediate phase of population growth at which the growth of central cells is impeded.

DISCUSSION: Our finding reveals that both global (soluble growth factors) and local (cell-cell interactions) mechanisms regulate epithelial population growth. The tight interplay between soluble growth factors and cell-cell interactions further drives the spatial heterogeneity in cell behaviors. An understanding of spatial dynamics of epithelial growth will offer strategies in determining design criteria for growth factor-releasing skin grafts.

CONTROLLING CARTILAGE SHEAR AND SLIDING IN VITRO FOR-MECHANOBIOLOGY AND TISSUE ENGINEERING

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INTRODUCTION: Articular cartilage is subject to both compression and shear during normal activities.¹ Compressive and shear^{2,3} loads have been applied to engineered cartilaginous tissue to improve its mechanical property and matrix synthesis. In shear tests, platens are apposed to cartilage tissue and displaced a fixed amount laterally, with the intent or assumption of a no-slip boundary condition. However, tissue responses may be due not only to the extent of shear deformation, but also to the sliding of the articulating surfaces, or both. To study the mechanobiology of native and engineered cartilage, it would be useful to identify articulating counter-surfaces that can impose distinct regimes of shear and sliding. The objectives of this study were to (1) develop a loading system that can apply a controlled amount of shear deformation and sliding to articular cartilage, and (2) to assess the effect of lubrication on this system.

METHODS: Samples: Cartilage disks were harvested from normal post mortem human talus joints ($\varnothing 3\text{mm}$), soaked in a lubricant bath, and measured for thickness. Experimental Design: Surfaces of impermeable polysulfone platens were either polished (P), mildly roughened (M), or roughened (R). Samples were tested separately for each platen type ($n=3$), first with PBS + protease inhibitors (PI), at 10, 20, and 30% compression, allowed to re-swell for 24h, and re-tested with normal bovine synovial fluid (SF) + PI. Mechanical Testing: Disks were placed in a custom chamber with a non-slip base connected to the horizontal actuator of Mach-1TM V500 mechanical tester (Biosyntech Canada). The articular surface was compressed at 0.5m/s and allowed to relax for 1/2h. At equilibrium, 20 cycles of 0 to +10% (of the unloaded thickness) lateral displacement was applied at 100 $\mu\text{m/s}$. Statistics: At all compression levels, peak shear stress (σ_{xz}) and maximum shear strain (Exz , peak strain prior to sliding) were calculated. Exz was calculated as the sum of strains determined incrementally from discrete data points of lateral load and position data files, averaged over 19 cycles after one preconditioning cycle. The effects of compression (10, 20, & 30%), lubricant (PBS, SF), and platen roughness (P, M, R) on σ_{xz} and Exz were analyzed by ANOVA and pair-wise comparison.

RESULTS: At each offset level, the equilibrium compressive stress was similar for different platens tested with either PBS or SF; also, compressive stress increased with increasing level of compression. Platen roughness had distinct effects on the regime of shear and sliding, as indicated by the lateral force response. Maximum σ_{xz} and Exz increased with increasing compression offset ($p<0.001$) and was higher for PBS lubricant ($p<0.05$), with an interactive effect ($p<0.001$). At 30% compression and for both lubricants, Exz reached the maximal (applied) strain for the rough platen. At 20% compression, Exz reached ~maximal strain with PBS as lubricant, and was higher for M and R platens with SF as lubricant ($p<0.05$). At 10% compression, differences in Exz due to platen roughness became marked and was significantly less for P than R platen ($p<0.05$).

DISCUSSION: These results describe a new type of biomechanical test system to control cartilage σ_{xz} and Exz , as well as sliding distance, for a specified lateral displacement. The ability to increase Exz above normal levels using roughened platens provides a way of studying the effects of supra-normal Exz , independent of the lubricant environment. The reduction in maximum σ_{xz} and Exz with the use of SF is consistent with its lubricating function.⁴ Thus, the lubrication properties of the bathing medium may affect, in certain regimes, the shear response of cartilage tissue in vitro. This biomechanical test system may be useful for studying cartilage responses to a variety of articulation stimuli and for applying controlled mechanical stimulation (i.e. compression and shear) to improve mechanical property of engineered tissues.

ACKNOWLEDGMENT: Supported by NIH.

REFERENCES: 1Hunziker+ OAC 2001. 2Hung+ ABME 2004. 3Waldman+ JBJS 2003. 4Schmidt+ OAC 2007.



POSTERS

Session I: Biomaterials Engineering

Session II: The California Triple Helix in Regenerative Medicine

Session III: Stem Cell Biology & Tissue Engineering

Session IV: Cytokines and Cell Signaling in Tissue Engineering

SYNTHESIS OF POLYMERS FOR MICRO- AND NANOPATTERNING OF ORIENTED PROTEINS

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The ability to immobilize proteins in specific orientations on surfaces is significant in applications where retention of bioactivity is essential. Herein, we describe an approach for patterning proteins on surfaces at the micro- and nanometer scale. We synthesized Boc-aminooxy tetra(ethylene glycol) methacrylate in two steps. A homopolymer and a copolymer with 2-hydroxyethyl methacrylate were then prepared by free radical polymerization. Films of these polymers were patterned by photoacid generator photolithography or electron beam lithography to produce micro- and nanoscale domains of aminooxy groups. These patterns were used to orient α -oxoamide modified proteins on the surface via oxime bonds. The synthesis of the monomer, polymer, and fabrication of the protein patterns will be discussed.

CONTROLLABLE BIOMIMETIC HYDROGEL SCAFFOLDS TO STUDY PULMONARY FIBROBLAST MECHANOTRANSDUCTION

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INTRODUCTION: The cause of idiopathic pulmonary fibrosis (IPF) is not well-understood but is thought to be the result of an abnormal wound-healing process in the lungs.¹ Mechanical injury has been shown to induce myofibroblast differentiation² but many patients with IPF do not present an injury. We are interested in the effect of mechanical stimuli on the activation of pulmonary fibroblasts. We are developing two- and three- dimensional synthetic cell culture environments where the local integrin binding and local mechanical environment can be independently altered for the study of pulmonary fibrosis.

METHODS: PEG hydrogels were synthesized using PEG (Mn = 575-10,000) diacrylate at 10wt% with 0-5 mM peptide using 0.05wt% photoinitiator (I2959). Adhesion peptides flanked with cysteines were incorporated into the hydrogel via reaction with the cysteines. The gels were sterilized and incubated with media prior to cell adhesion experiments. Human pulmonary fibroblasts (LL29) were seeded onto the gels at 50,000 cells/cm² in fresh media or encapsulated at 500,000 cells/gel. We measured adhesion up to 1 week using ATP and/or Picogreen and viability using LIVE/DEAD staining. Immunocytochemistry was performed to identify focal adhesions and α -SMA; confocal microscopy images were taken.

RESULTS: Cell seeding experiments on PEG4000 diacrylate with RGDS (2, 0.5, 0.05, 0.005 mM) showed that cell viability at 0.005 mM and 2 mM were comparable at 24 h. The cells adhered and spread to the substrate. Immunocytochemistry of fibroblasts seeded on PEG700 diacrylate with 0.5 mM RGDS at 8 days showed the presence of α -SMA which indicates the myofibroblast phenotype. For photoencapsulation of the fibroblasts in 0 or 0.5 mM RGD, there was no noticeable difference in cell viability at 3 days. At 10 days there are fewer dead cells in the gels with RGD.

DISCUSSION: This 2D system is a viable approach for the differentiation of pulmonary fibroblasts into the myofibroblast phenotype by using RGD peptide segments crosslinked into the PEG-diacrylate hydrogels. A concentration as low as 0.005 mM for RGDS on PEG4000 diacrylate gels promotes cell adhesion and spreading. The presence of α -SMA, a characteristic of myofibroblasts, shows the RGDS-functionalized PEG700 diacrylate gels promote fibroblast activation. This system is compatible with both 2D and 3D culture of pulmonary fibroblasts. We can vary the concentration and identity of the peptide epitopes independent of the network rigidity, which makes this system ideal for investigating mechanical signals in fibrosis.

REFERENCES: 1) Thannickal, V. J.; Toews, G. B.; White, E. S.; Lynch, J. P.; Martinez, F. J., Mechanisms of pulmonary fibrosis. Annual Review of Medicine 2004, 55, 395-417. 2) Morishima, Y.; Nomura, A.; Uchida, Y.; Noguchi, Y.; Sakamoto, T.; Ishii, Y.; Goto, Y.; Masuyama, K.; Zhang, M. J.; Hirano, K.; Mochizuki, M.; Ohtsuka, M.; Sekizawa, K., Triggering the induction of myofibroblast and fibrogenesis by airway epithelial shedding. American Journal of Respiratory Cell and Molecular Biology 2001, 24, (1), 1-11.

ENZYMATICALLY CROSSLINKED PROTEIN POLYMER HYDROGELS AS NOVEL TISSUE ENGINEERING SCAFFOLDS

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INTRODUCTION: Challenges in tissue engineering require the development of material systems that provide a temporary scaffold mimicking the natural extracellular matrix. Natural materials have good biocompatibility, but may have batch-to-batch variability and do not allow the manipulation of structures for tunable properties; although synthetic polymers are flexible in designing, they are polydisperse, and either may not be biodegradable or may degrade with toxic byproducts. Protein polymers with precisely tailored sequences can be synthesized by genetic engineering methods, which provide avenues to overcome these limitations. Herein, we created several random-coil protein polymers with functionalized block compositions that can be enzymatically crosslinked (via transglutaminase, TG) into hydrogels as tissue engineering scaffolds. These protein polymer hydrogels can be designed with properties specific for particular site implementation requirements for tissue engineering applications.

METHODS: The 'controlled cloning' [1] method was used to create a DNA template encoding the required amino acid sequences. The proteins were expressed, purified, and then characterized by MALDI, LCST, amino acid analysis and CD. Degradation studies were performed by incubating the protein polymers with trypsin and plasmin. TG crosslinked glu and lys residues on the protein polymer to form a hydrogel. The viscoelastic properties of the hydrogels were characterized by rheometry. The biological response of β -islet cells in the hydrogel scaffold was studied both in vitro and in vivo.

RESULTS: The DNA was designed and cloned successfully. The corresponding protein polymers with different lengths and block spacings were created, which were random coil and well soluble. They form hydrogels within a short time after adding TG. The protein polymers were biodegradable by proteases that cleave at lysine residues. The enzymatically crosslinked protein polymers behaved as a robust, elastic solid. The materials were biocompatible and can serve as a good scaffold for tissue engineering purposes, such as islet transplantation.

DISCUSSION: Through carefully designing the amino acid sequence, chain length, and block spacing, further modification on the protein hydrogels can be achieved to obtain a large extent of control over the mechanical, physicochemical and biofunctional properties.

REFERENCE: [1] J. Won, A. E. Barron, *Macromolecules* 2002, 35, 8281.

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SYNTHESIS OF PHOTOREACTIVE LINKERS WITH VARYING DEGRADATION RATES FOR USE IN BIOMEDICAL APPLICATIONS

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INTRODUCTION: A critical aspect of designing biomaterial carriers for cells and drug delivery is tuning and controlling the material's degradation behavior. Most synthetic biomaterials degrade via hydrolysis or enzymolysis. The rate of hydrolysis is pre-engineered and cannot be modified after the scaffold is fabricated under physiologically relevant conditions. The rate of enzymatic degradation is cell-mediated and is normally limited to local degradation. In the last decade, there has been considerable interest in using photochemistry to produce biomaterials because of the ability to form scaffolds in situ under physiological conditions¹. Integrating photochemistry as a degradation mechanism should be equally biocompatible, affording spatial and temporal control over the chemical, mechanical and physical properties of the biomaterial, and allowing for the controlled and triggerable release of therapeutic agents. We have designed a series of photodegradable linkers based on nitrobenzylether to provide a range of degradation rates upon exposure to long-wave UV light² (365 nm, biocompatible¹).

METHODS: The general synthetic procedure to produce the nitrobenzylether linkers is etherification of a phenol using ethyl-4-bromobutyrate, then aromatic nitration, followed by sodium borohydride reduction of the ketone/aldehyde, and finally hydrolysis of the ethyl ester. This scheme works well for the synthesis of molecules A and B, but we encounter limitations in the nitration step for molecules C and D. The synthesis of the fastest degrading groups (E and F) is modified because there are no phenolic groups on the ring.

RESULTS: Synthesis of molecules A and B has been successfully completed. Due to complications in the aromatic nitration step, synthesis of molecules C and D has not been completed. Molecule B degrades over the course of tens of minutes, so we expect rates closer to the minutes or seconds timescale for the faster degrading molecules. We have demonstrated controlled PEG-hydrogel network degradation and therapeutically relevant release of dexamethasone.

DISCUSSION: In contrast to photodegradable groups A and B, synthesis of the photolabile compounds C and D is more challenging due to lower reactivity towards nitration. The aryl ether groups both activate and direct the nitration step, and removing one aryl ether group from the ring results in a mixture of isomers. We are currently optimizing conditions for the synthesis and purification of C and D with specific regard to the nitration step.

REFERENCES:

1. Bryant, S. J.; Nuttelman, C. R.; Anseth, K. S. J. *Biomater. Sci.-Polym. E.* 2000, 11, (5), 439-457
2. Zhao, YR; Zheng, Q; Dakin, K; Xu, K; Martinez, ML; Li, WH. *J. Am. Chem. Soc.* 2004, 126, (14), 4653-4663.

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DETERMINATION OF RELATIONSHIP OF FIBRINOGEN AND THROMBIN CONCENTRATION TO FIBRIN SCAFFOLD PROPERTIES THROUGH THE USE OF DEXTRAN NANOPARTICLES

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INTRODUCTION: A chronic wound forms when any step of the wound healing process completes improperly. A potential treatment for this condition involves implantation of a fibrin scaffold seeded with fibroblasts into the wound site. In order for an implant to be successful, the pore size of the scaffold must be large enough to accommodate cellular nutrient exchange and proliferation, while not so large that the scaffold mechanical integrity is compromised. The purpose of this study was to determine the effects of different concentrations of fibrinogen and thrombin on scaffold porosity, diffusion capabilities, mechanical properties, and ultra-structure, using dextran nanoparticles as both scaffold probes and models of intracellular particles.

METHOD: Scaffolds of varying fibrinogen and thrombin concentrations were formed in modified Millipore™ Centricon® containers, and then evaluated for flow characteristics with serum-free DMEM with or without fluorescent dextran nanoparticles of varying sizes. Next, scaffolds were evaluated for the diffusive properties of dextran nanoparticles of varying sizes. Finally, scaffolds were tested for mechanical stiffness with an Instron machine, and ultrastructure with scanning electron microscopy.

RESULTS: Flow studies and SEM results demonstrated an inverse relationship between pore size and both fibrinogen and thrombin concentration, indicating that an increase in either fibrinogen or thrombin concentration results in a decrease in pore size and permeability.

DISCUSSION: These results suggest that higher concentrations of fibrinogen and thrombin inhibit nutrient uptake of seeded cells, confirming previous studies, which demonstrated a decrease in cell viability with an increase in fibrinogen concentration¹. Future studies involve evaluating dextran or other nanoparticles uptake by cells embedded in fibrin scaffolds.

REFERENCES: 1. Cox S, Cole M, Tawil B. "Behavior of human dermal fibroblasts in three-dimensional fibrin clots: Dependence on fibrinogen and thrombin concentration." *Tissue Engineering*, 2004; 10: 942-954.
2. Shu et.al. Modifications of flow measurement to determine fibrin gel permeability and the preliminary use in research and clinical materials. *Blood Coagulation and Fibrinolysis* 2005, 16:61-67

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CHITOSAN BASED INJECTABLE PARTICLES FOR BONE AND CARTILAGE REGENERATION

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INTRODUCTION: Chitosan (CS) is a linear polysaccharide consisting of (1-4) linked glucosamine and N-acetyl glucosamine. CS shares macromolecular properties with the natural extracellular matrices. The N-acetyl glucosamine moiety in CS is a structural features also found in the glycosaminoglycans (GAG). This analogous structure in CS is of great interest because a large number of growth factors and adhesion/receptor molecules bind to GAG. One interesting property of CS is its cationic nature and high charge density in solution. This allows the formation of insoluble ionic complexes with multivalent water-soluble anionic polymers under very mild conditions. In this study, tripolyphosphate (TPP), which is a non-toxic multivalent anionic, and chondroitin sulfate (CHS), which is one of the major GAG found in articular cartilage, were used as CS crosslinkers. Nell-1, osteogenic molecule, was incorporated, and release kinetics and bioactivity of Nell-1 from experimental CS formulations were characterized in vitro.

METHODS: Nell-loaded CS particles were prepared by ionic gelation methods. The association efficiency or release of Nell-1 from the CS particles was calculated using 3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde (CBQCA) protein assay. The bioactivity of the released Nell-1 from the CS particles was investigated by assessing its ability to increase the expression of alkaline phosphatase (ALP) from the human osteosarcoma cell lines (SaOS2).

RESULTS: The association efficiency of Nell-1 was around 70%. Nell-1 was released in phosphate buffered saline with a higher initial burst compared to the initial burst in water. This suggests that the presence of ions weakens ionic interactions, which increases swelling and delivery. The release of Nell-1 from the lyophilized Nell-loaded CS particles was also assessed. The release of Nell-1 from the lyophilized particles was not significantly different from the non- lyophilized particles. The incubating media collected from Nell-loaded CS particles or lyophilized Nell-loaded CS particles increased ALP activity of SaOS2, indicating that the biologic effect of Nell-1 was preserved during the incorporating procedure into CS particles. The association efficiency of Nell-1 increased in the presence of CHS as the second cross linker. The release of Nell-1 from CS/TPP/CHS particles was slower compared to CS/TPP particles. These systems are a promising scaffold in orthopedic applications.

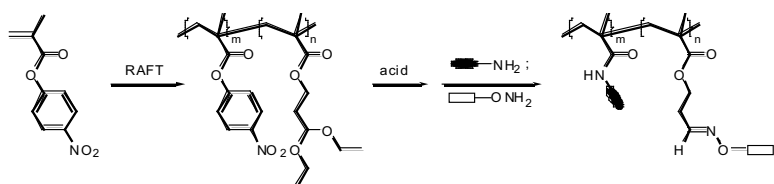
REACTIVE POLYMER SCAFFOLDS FOR BIOFUNCTIONALIZATION

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Well-defined bioactive polymers are important for therapeutic applications because they have predictable physical and pharmacokinetic properties. Polymers with narrow molecular weight distributions and defined architectures can be synthesized using controlled radical polymerization methods. The formation of reactive scaffolds by atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer (RAFT) polymerization and their subsequent biofunctionalization was undertaken. Polymers composed of reactive side chains provided a method to form biofunctional polymers. Poly(diethoxypropyl methacrylate) (pDEPMA) was synthesized by both ATRP and RAFT polymerization.² This polymer contained acetal side chains that hydrolyzed to reveal aldehydes. Reaction with O-hydroxylamine compounds resulted in oxime linked conjugates. Mono- and bisfunctionalization of this polymer was demonstrated. Another reactive polymer, poly(p-nitrophenyl methacrylate) (pNPMA), which contained activated esters, was synthesized by RAFT polymerization.² Functionalization occurred through substitution of the esters. The polymer was chain extended with DEPMA to form a pNPMA-b-pDEPMA block copolymer.³ Reaction of this macromolecule provided a foundation for facile preparation of diverse materials (Scheme 1).



Scheme 1. Synthesis and reaction of a block copolymer scaffold.

References

- (1) Li, R. C.; Broyer, R. M.; Maynard, H. D. "Well-defined polymers with acetal side chains as reactive scaffolds synthesized by atom transfer radical polymerization," *J. Polym. Sci., Part A; Polym. Chem.* 2006, 44, 5004-5013.
- (2) Hwang, J.; Li, R. C.; Maynard, H. D. "Well-defined polymers with activated ester and protected aldehyde side chains for bio-functionalization," *J. Controlled Release*, 2007, 122, 279-286.
- (3) Li, R. C.; Hwang, J.; Maynard, H. D., "Reactive Block Copolymer Scaffolds," *Chem. Commun.*, 2007, 3631-3633.

MEASURING THE DIFFUSION KINETICS OF PROTEINS THROUGH NOVEL MATRICES

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INTRODUCTION: Every year, 80,000 to 90,000 people in the United States are permanently disabled due to a Traumatic Brain Injury (TBI).⁶ Tissue engineering is currently being studied as a method to combat TBI. This process involves expanding stem cells from a biopsy and cell culturing on a temporary three-dimensional scaffold. ^{2,4,5} Growth factors then enable the cells to differentiate into neurons. The rate of release of growth factors then depends on the degradation rate of the scaffold as well as the pore size, connectivity, and tortuosity.^{1,3} The diffusion profile gives valuable information about the environment for cells. In this study, we investigated the diffusion of bovine serum albumin (BSA), a protein that is similar to the growth factors of interest, out of collagen and chitosan scaffolds.

METHODS: Dish tests were developed to determine the diffusion kinetics out of the scaffolds. The dish test involved immersing the scaffold in an approximately infinite medium. These scaffolds included collagen, chitosan and a collagen-chitosan composite, which were prepared from liquid substrate by lyophilization. The proteins to be tested included Bovine Serum Albumin (BSA), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and basic fibroblast growth factor (bFGF). Proteins were added at various stages during sample preparation to compare diffusion profiles. The Biorad protein assay was used to measure the BSA concentration during diffusion; similarly an Enzyme Linked Immunosorbent Assay (ELISA) was used to measure growth factor concentration.

RESULTS: Effective diffusivity values were calculated using Fick's Diffusion Laws assuming one dimensional flow across a thin film with uniform initial concentration and infinite sink conditions. For collagen scaffolds, the diffusivity was higher when protein was added after lyophilization (rather than before) although this may have been due to the presence of unknown amounts of BSA in cell media. The chitosan scaffolds were never successfully gelled despite numerous attempts.

DISCUSSION: BSA diffuses out of collagen hydrogels much more quickly when it is added after lyophilization. The diffusivity for the collagen sample with BSA in the first swell (before freeze drying) was significantly less than the diffusivity for the hydrogel with BSA in the second swell (after freeze drying). Although the difference in diffusivities may be from the difference in sample preparation, it may also be due to the presence of cell media in the swelling steps of the collagen with BSA in the second swell. The data shows that the steady-state concentration is 40 !g/mL. This value is twice as high as expected given the initial concentration of 20 !g/mL BSA in the hydrogel. Currently, more collagen and chitosan samples are being produced and prepared with nerve growth factor (NGF).

REFERENCES:

1. Babensee, J.E., McIntire, L.V., Mikos, A.G. "Growth Factor Delivery for Tissue Engineering." *Pharmaceutical Research*. 17 (2000): 497-504.
2. Chen, G., Ushida, T., Tateishi, T. "Scaffold Design for Tissue Engineering." *Macromolecular Bioscience*. 2 (2002): 67-77.
3. Lee, M., T.T. Chen, M.L. Iruela-Arispe, B.M. Wu & J.C. Dunn. "Modulation of protein delivery from modular polymer scaffolds." *Biomaterials*. 28 (2007): 1862-1870.
4. Levenberg, S., N.F. Huang, E. Lavik, A.B. Rogers, J. Itskovitz-Eldor & R. Langer. "Differentiation of human embryonic stem cells on three-dimensional polymer scaffolds." *Proceedings of the National Academy of Sciences of the United States of America*. 100 (2003): 12741-12746.
5. Salvay, David, Shea, Lonnie. "Inductive tissue engineering with protein and DNA-releasing scaffolds." *Molecular BioSystems*. 2 (2006): 36-48.
6. Traumatic Brain Injury in the United States. January 2006. Centers for Disease Control and Prevention. 25 May 2007
<http://www.cdc.gov/ncipc/pubres/TBI_in_US_04/TBI%20in%20the%20US_Jan_2006.pdf>

FIBRIN AND BIOMIMETIC APATITES FOR BONE REGENERATIONTsang EJ¹, Duong H¹, Tawil B¹, Wu BM¹¹University of California, Los Angeles,
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ABSTRACT:

The American Association of Orthopedic Surgeons predicts a “Decade of Orthopedics” and unprecedented orthopedic needs due to the active lifestyles of the aging baby boomers. There is a significant need to develop off-the-shelf biomaterials for delivering autologous stem cells to regenerate lost tissues due to trauma, disease, and surgical resection.

Fibrin is a natural biopolymer that stabilizes blood clot and assists in the wound healing process. With a long history of human safety as hemostatic and adhesive agents, fibrin provides a convenient carrier for cells, growth factors, and other materials. Our laboratory has previously reported that fibrin formulation is a critical factor on human mesenchymal stem cell (hMSC) proliferation [1]. We also observed minimal osteogenic differentiation by the hMSCs. The objective of this project is to enhance the osteogenicity of fibrin formulations, and investigate cell signaling within the experimental formulations.

COMBINATION ELECTROSPINNING OF POLYGLYCOLIC ACID WITH SUCROSE GENERATES HIGH POROSITY SCAFFOLDS

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BACKGROUND: Electrospinning is a well established method to fabricate scaffolds with nanofibers and highly interconnected pores. The typical setup consists of a collector, a syringe pump with needle tip containing a polymer solution and a high voltage power supply. The application of an electrical field creates a charge within the polymer solution, the electrostatic force overcomes the surface tension of the solution and this produces thin polymer fibers. Resulting scaffold materials typically have pore sizes of $< 10 \mu\text{m}$ which limits their usefulness.

HYPOTHESIS: Electrospinning of a polymer solution in combination with a filler material may be a method to fabricate tubular scaffolds with larger pore sizes.

METHODS: A 10 wt.% PGA solution was dissolved in HFIP and dispensed through an 18g needle with a syringe pump at a flow rate of 1 ml/h. A 5 mm thick copper rod collector was placed at a distance of 8 cm from the needle tip and rotated at 1200 rpm. Electrospinning was performed applying an electrical field of 28 kV. A second solution of 70 wt.% sucrose in 70% ethanol was electrospun with a flow rate of 10 ml/h and a collector- needle- distance of 15 cm. PGA and sucrose solutions were spun alternating at time intervals ranging from 2- 10 min. PGA/sucrose tubes were leached in distilled water for 15 min and dried in the hood under constant airflow. SEM images of tube sections were obtained before and after leaching to determine pore sizes.

RESULTS: This technique of serially electrospun PGA and sucrose results in a very compact bioscaffolding made of a strong PGA fiber mesh and crystalline sucrose particles. Leaching of sucrose left pores of 50 - 200 μm . Pore size could be controlled by varying the time interval of each spinning sequence. The longer the electrospinning time for the sucrose solution the greater the porosity.

CONCLUSION: Combination electrospinning represents an excellent technique to generate designer scaffolds with controllable, large pore sizes.

AN ASSAY SYSTEM FOR EARLY DETECTION OF PERMEABILITY OF HUMAN “ENDOTHELIUM”

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The molecular mechanisms by which endothelial permeability occurs are not well understood. Such mechanisms are studied more readily *in vitro*, underscoring the importance of using systems that mimic human endothelium *in vivo*. Here, we present an assay that better models human endothelium by using primary human microvascular endothelial cells (hMVEC), because permeability primarily occurs at the microvascular level, and transwell filter units coated with matrigel, extracellular matrix that mimics basal lamina, the matrix that is tightly associated with endothelium and is critical for its proper function. As a tracer molecule, we used 3kDa dextran-FITC to detect leakage through very small gaps that are present in the early stages of permeability. The permeability-inducing agents VEGF and thrombin were added to the lower chamber of the transwell units to mimic inflammatory conditions *in vivo*. After optimization, we were able to minimize basal permeability and to detect rapid changes in permeability stimulated by thrombin and VEGF, similar to that observed *in vivo*. This system can be used to better understand the molecular mechanisms of human vascular permeability in a more *in vivo*-like setting, and thus may be used to test effective therapeutics to prevent and treat diseases involving persistent permeability .

BUILDING OF A HUMAN ARTERY MODEL SYSTEM FOR STUDIES OF EARLY PROCESSES IN ATHEROSCLEROTIC PLAQUE DEVELOPMENT

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Cardiovascular disease is the leading cause of death in many countries, especially in the western world. Development of a good model system will allow the study of cell-cell and cell-extra cellular matrix (ECM) interactions, and to determine the effects of drugs on the blood vessel biology. Because human blood vessels are difficult to obtain, it is very useful to build a blood vessel system for studies in vitro to understand cardiovascular disease. Current methods often use the cells cultured in 2D on plastic culture dishes. However, this system does not reflect well conditions in vivo where the cells are in a 3D environment, in many cases in direct contact with a complex ECM. In order to alleviate this problem, we constructed a 3-D blood vessel culture system that mimics an aorta blood vessel wall, using human endothelial cells, ECM and smooth muscle cells. This tissue was built using transwell systems to allow for the tissue to be exposed to the culture media from both sides and consists of a thick layer of human aortic smooth muscle cells embedded in a small amount to ECM molecules overlaid by a tight monolayer of human aortic endothelial cells. Using a variety of cell, molecular and histological approaches we show that in this system has many of the characteristics of an arterial wall. Labeling for peripheral endothelial cell adhesion molecule (PECAM), a specific marker for endothelial cells that is critical for maintaining endothelial cell-cell adhesions, shows a single layer of endothelial cells. Labeling for Col IV, a molecule found almost exclusive in basal lamina, shows a continuous lamina underlying the endothelial cells. Labeling for SMA shows that the smooth muscle cells are well packed into a thick multi-layered mimicking the tunica media. This “blood vessel wall” culture system also undergoes a curvature, mimicking of a blood vessel, to make the cells not only in a molecular system that approximates the situation in vivo, but also may be exposed to mechanical stresses. To show that this system responds to stimulatory agents we exposed it to a cigarette smoke solution that contains many of the toxicants that a person who smokes is exposed to, and treated the model system. We found that the endothelial layer is adversely affected and that monocytes are more prone to undergo transendothelial migration and lodge in between the smooth muscle cell layer. When exposed to the smoke solution the cells also express increased level of molecules that are important in the inflammatory state, such as IL-8, MCP-1, and VCAM-1. In addition when exposed also to Oxidized LDL the smoke stimulates what appears to be aggregation of macrophages, which could represent initiation of plaque. These result show that the arterial wall model we have developed can be useful to study early events in atherosclerotic plaque development.

PATTERN FORMATION IN PHYSIOLOGY AND PATHOPHYSIOLOGY: USING TISSUE SELF-ENGINEERING

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Morphogenesis, the formation of spatial patterns, is an integral part of embryological development, and is also important in the formation of pathological patterns later in life. The mechanisms by which patterns form are, in general, poorly understood. Our group uses mathematical models and computer simulation to understand the biochemical and spatial processes that create particular kinds of pattern.

These mathematical models use Partial Differential Equations (PDEs) to describe the diffusion and reaction of morphogens, proteins that act as activators or inhibitors of cell movement and/or commitment. Their biological and biochemical interactions, together with physical diffusion processes, can account for a number of physiologically important patterns.

For example, in atherosclerotic calcification, calcified lesions form 'focally,' that is, as localized spots. Bone morphogenetic protein (BMP), a potent chemotactic agent and inducer of commitment to bone phenotype, is known to be active in these calcifying lesions, along with several inhibitors of BMP, such as matrix GLA protein (MGP). Using a PDE model of BMP and MGP, we showed that 'stripe' and 'spot' patterns could be explained. Understanding how the spatial mechanisms produce the pattern also enabled us to make interventions to alter the patterns. Other potential examples of BMP-driven spatial patterns include the transition from cortical to trabecular bone, and the formation of other types of localized lesions, such as on the skin. Many other morphogens are now known to be physiologically active, including FGF, EGF, retinoic acid, squint and others.

The mathematical models make possible a type of tissue engineering that uses the inherent spatial dynamics, the tissue "self-engineering" of the cells themselves, to enhance and guide our interventions.

A NEW GENERATION ORGAN CULTURE ARISING FROM CROSS-TALK BETWEEN MULTIPLE PRIMARY HUMAN CELL TYPES

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The inability to experiment directly on humans strongly constrains biomedical research, creating a great need to develop cultures that mimic human tissues and organs as experimental systems that can be used to directly understand and manipulate biological processes. The advent of availability of primary human cells now makes possible engineering of such organ cultures. Here we report the generation of a human “skin”, arguably the simplest human tissue. Beginning with three primary cell types taken from adult tissues, this organ culture develops into a mature tissue containing a stratified epithelium and an interconnected network of mature microvessels, with appropriate matrix molecules and cytokines. Surprisingly, pericytes and monocytes appear adjacent to and within “blood” vessels, respectively. These cultures respond appropriately to stimulators of specific biological processes, providing a vehicle to investigate basic biological processes such as: (i) cell-cell and cell-microenvironment interaction; (ii) transdifferentiation of one cell type to another and/or differentiation from stem cells present in adult tissues; (iii) opportunities for genetic manipulation of human tissues to understand function. Moreover, this “skin” can potentially be developed into a tailored “living bandage” for patients with impaired healing and can serve as prototype for the development of other human organ cultures.

CHEMOKINE SECRETION BY SENESCENT FIBROBLASTS AND CHRONIC WOUNDS

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Following acute injury to the skin there is a rapid influx of inflammatory cells, beginning with neutrophils, which occurs within hours. The initial signal results from platelet degranulation, which releases platelet basic protein that is cleaved to give connective tissue activating protein (CTAP) and the NAP-2 series of neutrophil chemoattractants. This bolus release initiates neutrophil recruitment, but recruitment is sustained by cellular production of neutrophil chemoattractants, particularly by keratinocytes and fibroblasts. In the case of fibroblasts CXCL1, CXCL5, CXCL6 and CXCL8 are induced at the gene level and secreted. However, fibroblasts isolated from chronic wounds, and also from skin prone to chronic wounds, show many senescence-like features such as flattened morphology and poor growth. Similar properties, known as replicative senescence, can be induced in fibroblasts by 18 – 22 rounds of subculture in vitro. We have observed that, as fibroblasts approach senescence in this system, the production of the neutrophil chemoattractant chemokines declines. If the phenotypically similar fibroblasts in chronic wounds also fail to maintain neutrophil recruitment sufficient to prevent bacterial colonization, then contaminating bacteria would become established and form communities termed biofilms. When established as a biofilm, bacteria change gene expression, become resistant to further attack by neutrophils and other components of the innate immune system. They are also resistant to antimicrobial intervention and may persist in the wound site in a manner that does not cause a clinical infection.

Chronic wounds fail to reepithelialize and heal. In an investigation of possible mechanisms that might inhibit keratinocyte migration in chronic wounds we found that there was a direct effect of bacterial products. Lipopolysaccharide prevented keratinocyte migration in an in vitro scratch assay. Half maximal inhibition occurred at a concentration of less than 1 ng/mL. This concentration roughly corresponds to the concentration expected from the normal skin flora. Addition of neutralizing antibodies to the toll-like receptors, TLR 2 and TLR4, reduced inhibition by 25% and 60% respectively, indicating that these receptors are involved in the mechanism of inhibition.

These results are consistent with the hypothesis that inadequate secretion of neutrophil chemoattractant chemokines by fibroblasts expressing a senescence-like phenotype leads to failure to prevent bacterial contamination and colonization of an acute wound. Biofilms become established and their products directly inhibit keratinocyte migration. They may thus be important in preventing the reepithelialization of chronic wounds and their healing.

TRANSACTIVATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-2 (VEGFR2) BY INTERLEUKIN-8 (IL-8/CXCL8) IS REQUIRED FOR IL-8/CXCL8-INDUCED ENDOTHELIAL PERMEABILITY.

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Interleukin-8 (IL-8/CXCL8) is a chemokine that increases endothelial permeability during early stages of angiogenesis. However, the mechanisms involved in IL-8/CXCL8-induced permeability are poorly understood. Here, we show that permeability induced by this chemokine requires the activation of Vascular Endothelial Growth Factor Receptor-2 (VEGFR2/Fik-1/KDR). IL-8/CXCL8 stimulates VEGFR2 phosphorylation in a VEGF-independent manner suggesting VEGFR2 transactivation. We investigated the possible contribution of physical interactions between VEGFR2 and the IL-8/CXCL8 receptors leading to VEGFR2 transactivation. Both IL-8 receptors interact with VEGFR2 following IL-8/CXCL8 treatment, and the time course of complex formation is comparable to that of VEGFR2 phosphorylation. Src kinases are involved upstream of receptor complex formation and VEGFR2 transactivation during IL-8/CXCL8-induced permeability. An inhibitor of Src kinases blocked IL-8/CXCL8-induced VEGFR2 phosphorylation, receptor complex formation, and endothelial permeability. Furthermore, inhibition of the VEGFR abolishes RhoA activation by IL-8/CXCL8, as well as gap formation, suggesting a mechanism whereby VEGFR2 transactivation mediates IL-8/CXCL8-induced permeability. These studies point to VEGFR2 transactivation as an important signaling pathway utilized by chemokines such as IL-8/CXCL8, and may lead to the development of new therapies that can be used in conditions involving increases in endothelial permeability or angiogenesis, particularly in pathological situations associated with both IL-8/CXCL8 and VEGF.

AUTOMATED QUANTITATIVE ANALYSIS OF EPITHELIAL CELL SCATTER

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INTRODUCTION: Epithelial-mesenchymal transition (EMT) is a mechanism by which epithelial cells transform into a more migratory phenotype, resembling fibroblasts. EMT is important for the formation of multicellular structures during embryonic and adult development, but also functions in tumor invasion and metastasis. Epithelial cell scatter in vitro closely parallels events that occur during EMT in vivo, including the disruption of cell-cell adhesions and the acquisition of a motile phenotype. This allows cells to escape the epithelial layer and invade the surrounding space. A key limitation in our understanding of cell scatter is that the resulting phenotypic changes are largely characterized in only qualitative terms.

METHODS: To better understand the quantitative contributions of molecular signals to scatter, we have developed a panel of metrics that capture both the early and late phases of scatter. These metrics were measured using time-lapse video microscopy of non-tumorigenic epithelial cells that stably express both nuclear- and membrane-localized fluorescent proteins. Automated image processing techniques to track cells in fluorescent images were developed using MATLAB.

RESULTS: We observe that cell scatter is controlled by multiple soluble factors. In MCF-10A cells, for instance, epidermal growth factor (EGF), cholera toxin and insulin synergistically affect both cluster size and inter-nuclear distance. With pharmacological inhibitors and DNA constructs, we are parsing the mechanisms that direct both the breakdown of intercellular adhesions and cell motility.

DISCUSSION: Taken together, these studies provide broadly applicable quantitative image analysis techniques and insight into the control of epithelial cell scatter, both of which will contribute to the understanding of EMT and metastasis.

REDUCING CORNEAL HAZE BY AN ENDOTHELIAL/KERATOCYTE COCULTURE METHOD

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INTRODUCTION: In a healthy cornea, the primary cell type in the stroma is the quiescent keratocyte, which contributes to the cornea's transparency by expressing aldehyde dehydrogenase class 1 (ALDH1) and transketolase (TKT) (Jester, 1999). When the cornea is wounded or grown in vitro, the keratocytes differentiate to repair fibroblasts and thus upregulate α -smooth muscle actin (α -SMA) and downregulates ALDH and TKT. This differentiation from keratocyte cells into fibroblasts and myofibroblasts causes the cells to appear fibroblastic, and leads to corneal haze contributing to a loss of transparency (Berryhill, 2002). Previous studies in our lab have found that endothelial/keratocyte co-cultures can delay the onset of the fibroblastic phenotype. This study investigated the effects of monolayer endothelial-keratocyte co-culture on fibroblast expression of proteins α -SMA, TKT, and ALDH1 at days 2, 3, 4, 5, and 7.

METHODS: Corneal stromal and endothelial cells isolated from New England Albino rabbits were grown in co-culture (Figure 1) and stromal cells alone were grown in monolayer. Fibroblasts co-cultured with endothelial cells were compared to fibroblasts in three monolayer conditions including normal media (NM), $\frac{1}{2}$ normal $\frac{1}{2}$ endothelial media (NMEM), and TGF- β media (TGF- β) for 7 days. Samples were analyzed by western blot for expression of α -SMA, TKT and ALDH1A1 on days 2, 3, 4, 5, and 7, and were normalized to GAPDH loading control after densitometric analysis.

RESULTS: Western Blotting and densitometric analysis validated that TGF- β upregulates α -SMA and demonstrated that levels of α -SMA remained low in co-culture until day 4, which may suggest that co-culture delays differentiation from myofibroblastic to quiescent keratocyte phenotype.

DISCUSSION. Our results demonstrate that endothelial/keratocyte co-culture have a potential to control and delay the myofibroblastic differentiation. Experiments are ongoing to determine the true reversion of the fibroblastic to the quiescent keratocyte phenotype as indicated by TKT and ALDH expression.

OCULAR FAT DERIVED ADIPOSE STEM CELL LINES EXPRESS DEPOT DEPENDENT DIFFERENCES IN PROTEIN EXPRESSION AND DIFFERENTIATION POTENTIAL.

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INTRODUCTION: Ocular injury and disease account for over 50 billion dollars annual health care cost in the United States, and thus, represents a major opportunity for a stem cell based tissue engineered solution. Adipose tissue is well known to contain cell populations with regenerative properties both in vitro and in vivo. In particular the adherent culture expanded cells, referred to as adipose derived stem cells (ADSCs) have been demonstrated to differentiate in vitro toward multiple mature cell phenotypes such as adipocytes, osteoblasts, chondrocytes, smooth and striated muscle, endothelium, hepatocytes, neurons and glial cells. In addition these cells are known to secrete immunomodulatory, anti-apoptotic, vascular promoting, and other growth factors that have efficacious roles in wound repair and tissue regeneration. To date no significant differences in the differentiation potential of ADSCs obtained from different subcutaneous adipose tissue depots have been observed.¹ The ocular fat pads, however, are known to have different developmental origins (mesoderm versus neural crest). We hypothesized that ADSC cell lines established from the neural crest derived ocular adipose may have unique characteristics that could make them suitable for use in treatment of ocular disease and wound repair. These studies detail the characterization of 4 novel ocular ADSC cell lines.

METHODS: Human orbital fat was obtained during routine eyelid surgery, homogenized, enzymatically digested and enriched for ADSCs by plastic adherence from four donors. Cell lines derived from different ocular depots were established in culture for 2-3 passages and then assayed for CD cell surface marker protein expression and differentiation potential toward, adipocytic, neuro/glial and smooth muscle phenotypes.

RESULTS: Four cell lines were extensively characterized. Two from nasal depot derived tissues and two from central depot derived tissues. All cell lines were able to undergo differentiation into adipocytes, smooth muscle progenitors and neuro/glial progenitors based upon oil red O staining, smooth actin immuno-staining and immunofluorescent staining for type III β -tubulin, NeuN, GFAP, and O4 protein expression, respectively. CD marker expression was two fold different in lines derived from different depots for CD34 and CD90 but not for CD11, CD31, CD45, or CD105.

DISCUSSION: This study identifies for the first time cells in different ocular adipose depots capable of multipotential differentiation. Furthermore, retention of higher CD90 and CD34 marker protein expression through multiple cell passages suggests that the neural crest derived ADSC cell lines possess a novel phenotype which may be beneficial for their use as a cell source in tissue engineering based ocular therapies.

REFERENCES: [1] Strem BM, Hicok KC, Zhu M, Wulur I, Alfonso Z, Schreiber RE, Fraser JK, Hedrick MH. Multipotential differentiation of adipose tissue-derived stem cells. *Keio J Med.* 2005 Sep;54(3):132-41

OSTEOARTHRITIC CELLS FOR HUMAN CARTILAGE TISSUE ENGINEERING

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INTRODUCTION: Treatment of osteoarthritis (OA) by tissue engineering represents a major and increased challenge compared to treatment of small focal cartilage defects. Since cell sources from normal tissue for tissue engineering can be limited, [1,2] and the recipient joint is that afflicted by OA, an ideal alternative would be to utilize cells derived from cartilage of OA joints. The hypothesis of this study was that cells derived from OA cartilage can be expanded and redifferentiated and thereby be considered a cell source for human cartilage tissue engineering.

METHODS: Cell Isolation: OA cartilage was obtained from patients undergoing total knee arthroplasty (n=7) and digested to obtain cells. [6] Exp.I. Cell Expansion: Cells were seeded in monolayer and incubated for 21 days in media with fetal bovine serum (FBS) or human serum (HS) \pm TGF- β 1, FGF-2, and PDGF-bb (TFP). [3-5] Exp.II. Cell Redifferentiation: Expanded cells were released from monolayer, encapsulated in alginate, [7] and incubated for 14 days in media with supplements (dexamethasone, ITS+1, TGF- β 1, FBS, HS, and adult bovine serum) comprising 14 conditions. Biochemical Analysis: Samples were analyzed for DNA [8] and sulfated glycosaminoglycan (sGAG). [9] Histology: Cells and their pericellular matrix were stained with alcian blue. [10] Statistical Analysis: ANOVA was used to analyze effects with medium and TFP as fixed factors and patient as a random factor with Tukey post-hoc testing.

RESULTS: Cell Expansion: TFP markedly stimulated cell growth ($p < 0.001$). There was stimulation by serum at 2% or 5% ($p < 0.05$), but no detectable difference between serum concentrations or types (HS vs. FBS). A ~100-fold expansion was achieved with serum + TFP conditions within a 17 day period. Cell Redifferentiation: Medium had a significant effect ($p < 0.001$) on sGAG/cell. Overall, sGAG/cell was higher in medium containing serum. Alcian blue staining indicated some differences in the CM between groups, even amongst those where sGAG/cell was similar. In the presence of HS, cells formed chondron-like clusters.

DISCUSSION: The current results indicate that OA cartilage is promising as a source of cells that can both proliferate and redifferentiate. HS appeared unique in its induction of formation of chondron-like structures, similar to those in native cartilage. [11] Overall, the results suggest that OA cartilage may serve as a viable cell source for large scale engineering of cartilage tissue.

REFERENCES: [1] Hunziker OAC 10:432, 2001. [2] Giannoni+ CTO 184:1, 2006. [3] Tallheden+ Arth Res Ther 7:560, 2005. [4] Barbero+ OAC 12:476, 2004. [5] Francioli+ Tiss Eng 13:1227, 2007. [6] Kuettner+ J Cell Bio 93:743, 1982. [7] Masuda+ JOR 21:139, 2003. [8] McGowan+ OAC 10:580, 2002. [9] Farndale+ BBA 883:173, 1986. [10] Sun+ JOR 4:427, 1986. [11] Poole J Anat. 191:1, 1997.

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REGULATION OF SUPERFICIAL ZONE PROTEIN (SZP), A BOUNDARY LUBRICANT IN THE DIFFERENT TISSUE COMPARTMENTS OF THE KNEE JOINT: IMPLICATION FOR TISSUE ENGINEERING

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INTRODUCTION: SZP, a 345-kDa proteoglycan and a key mediator in boundary lubrication of diarthrodial joints, is synthesized by chondrocyte in the superficial zone of articular cartilage and synovial lining cells. Recently, SZP has also been identified in meniscus, and ligament. TGF- β has been shown to increase the expression of cartilage- and synovium-derived SZP, whereas IL-1 conversely causes down-regulation of SZP expression. In the current study, we examined the effects of TGF- β 1 and IL-1 β on SZP protein and mRNA expression by four compartments of intraarticular tissues, namely, the superficial zone of articular cartilage, synovium, meniscus, and anterior/posterior cruciate ligaments (ACL/PCL).

METHODS: For explant culture, full-thickness cartilage plugs, synovium, meniscus, and PCL were obtained from calf stifle joints. For monolayer culture, these tissues were digested with collagenase. Explants and Isolated cells were cultured in serum-free chemically defined medium with either TGF- β 1 or IL-1 β . Sections from cultured explants were immunolocalized for SZP using mAb S6.79. Accumulation of SZP in the culture medium was quantified by ELISA. RNA was extracted for quantitative RT-PCR analysis using primers specific to bovine SZP.

RESULTS: Immunohistochemistry revealed that explants treated with TGF- β 1 for 3 days exhibited increased signal intensity of SZP at the surface. In cartilage, synovium, and PCL explants, the localization extended more deeply. Conversely, treatment of the explants with IL-1 β resulted in a decreased signal intensity of SZP at the surface and reduction in SZP retained within the tissues. In the control monolayer culture, the SZP level in the culture medium was much lower in meniscus-derived cells and ACL/PCL-derived cells compared to synovial cells and superficial zone chondrocytes. TGF- β 1 stimulated SZP accumulation in all four kinds of cells. A significant dose-dependent increase in SZP protein was observed. In contrast, treatment with IL-1 β significantly reduced SZP protein levels. The expression of SZP mRNA was dramatically enhanced by TGF- β 1, whereas IL-1 β inhibited SZP expression.

DISCUSSION: These findings demonstrated that SZP secretion and expression by the intraarticular tissues are precisely regulated by TGF- β 1 and IL-1 β , and may have implications for tissue-engineering strategies that aim to replicate the lubricant function of bioconstructs formed in vitro. Modulation of SZP expression by growth factors and cytokines could enable investigators to engineer functional bioconstructs of articular cartilage, meniscus, and ligaments.

SMOOTH MUSCLE DIFFERENTIATED ADIPOSE DERIVED STEM CELLS SHOW CONTRACTILE RESPONSES TO PHARMACOLOGIC AGENTS MEASURED WITH A COLLAGEN GEL SYSTEM

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INTRODUCTION

Adipose derived Stem Cells (ASC) had been shown to be pluripotent, possessing the capacity to differentiate into neurogenic, osteogenic, adipogenic, myogenic and cardiomyogenic lineages in vitro. Our group has recently shown that they can also differentiate into smooth muscle cells. Healthy, functional smooth muscle cells in abundance are in demand clinically for cellular replacement therapy and tissue engineering purposes to repair smooth muscle organs. ASCs hold promise as an excellent source of smooth muscle cells since they are autologous and highly abundant, but their functional contractility was largely unknown.

METHODS

Experiments were designed to show functional contractility of the differentiated ASC-smooth muscle cells (ASC-SM). To do so, cells were mixed in a soft 3D collagen gel laced with glass beads as trackers in which the contractile response of the cells to drugs can be visualized by 1) the dimensional changes of the collagen gel; and 2) the changes in distance between the glass bead trackers dispersed within the gel. The receptor-specificity of the contraction was tested by using 1) the agonist of a specific cell-surface receptor alone; and 2) pre-blocking of the receptor with an antagonist, followed by the agonist. Differences in contractile response were tested with 4 cell types: 1) ASC-SM; 2) undifferentiated ASC; 3) rat bladder smooth muscle cells; and 4) human T-lymphocytes.

RESULTS

ASC-SM showed similar contractile response to carbachol as native smooth muscle cells harvested from rat bladders, while the undifferentiated ASC and human T-lymphocytes showed minimal contraction. Moreover, the degree of contraction was drug dose-dependent, where the contraction increases with increasing agonist concentration and plateaus beyond a certain threshold. Lastly, results suggest that ASC-SM's contractility was receptor-specific, where the contractility was dramatically abolished when the receptors were pre-blocked with the antagonist before being treated with the agonist.

DISCUSSION

The glass-bead-laced collagen gel system is a useful tool to study cell contraction to various pharmacologic agents. ASC-SM's contractility is cell type-specific, dose-dependent and receptor-specific.

ORIGIN OF PERIENDOTHELIAL CELLS IN MICROVESSELS DERIVED FROM HUMAN MICROVASCULAR ENDOTHELIAL CELLS
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*** EQUAL CONTRIBUTION**

In microvessels, periendothelial cells expressing alpha smooth muscle actin (α SMA) interact with the endothelial cells and are essential for vessel maturation and stabilization. In adult tissues, the cellular origin of the periendothelial cells is still not clear, in particular in humans. To determine the origin of human periendothelial cells, we used a recently-developed 3D co-culture system that mimics human skin connective tissue. This system is composed of normal human dermal fibroblasts (NHDF), human dermal microvascular endothelial cells (HMEC-1), and a collagen matrix. In this system, “microvessels” composed of an endothelial lumen with associated periendothelial cells develop. Using this co-culture system, we (i) labelled fibroblasts with the vital dye CFDA-SE, cultured them with unlabeled endothelial cells, and observed that only endothelium-associated CFDA-SE-labelled cells express α SMA; (ii) infected endothelial cells with a retrovirus stably expressing eGFP, cultured them with unlabeled fibroblasts, and observed that cells expressing α SMA did not co-express eGFP, but were associated with the eGFP-expressing endothelial cells of the microvessels; (iii) show that the periendothelial cells communicate with the endothelial cells via junction complexes and (iv) show that the periendothelial cells are included within the basal lamina of the microvessels. Together, these results indicate that periendothelial cells arise by differentiation from fibroblasts and that they require interaction with endothelial cells to undergo that differentiation.

THE ROLE OF UCB IN BONE AND CARTILAGE DEVELOPMENT - POTENTIAL IN CHONDROGENESIS

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INTRODUCTION: UCB is an 810-amino acid secreted protein that is overexpressed in the prematurely fused sutures of craniosynostosis patients. Transgenic mice that overexpress UCB also exhibit premature suture fusion. Conversely, ENU-induced UCB-null mice with induced point mutation and truncated UCB protein suffer from defective bone and cartilage development. We observed significant differences in endochondral ossification in UCB-null mice, suggesting either a defect in chondrocyte differentiation or an increase in chondrocyte proliferation.

Inflammation and degeneration of cartilage results from age and traumatic injury. Therefore, tissue engineering of cartilage is of significant clinical and commercial interest. A common approach to cartilage repair involves the isolation, expansion, and re-implantation of autologous chondrocytes from a small biopsy. The primary drawback of harvested chondrocytes is their propensity to dedifferentiate in vitro; as early as one passage in vitro, such cells tend to lose the characteristic phenotype of chondrocytes. This in turn may limit their ability to properly synthesize and secrete important elements of the cartilage matrix, including proteoglycans and collagen, when implanted in vivo. The goal of this project is to evaluate the feasibility of using UCB to overcome chondrocyte dedifferentiation.

METHODS: Goat auricular chondrocytes were harvested and transduced with one of 3 adenoviruses: Group 1 - LacZ (AdLacZ), Group 2 - UCB (AdUCB), and Group 3 - BMP-2 (AdBMP-2). Transduced chondrocytes were injected subcutaneously in F127 pluronic gel into nude mice. The presence of cartilage was investigated at 4 weeks by histology, microCT, and immunohistochemistry.

RESULTS: Histological analysis revealed that UCB promotes chondrogenic differentiation and cartilage formation. Immunohistochemistry revealed positive type X collagen, tenascin-C, and phospho-ERK staining in UCB groups. In contrast, BMP-2 induced formation of small cartilage islands and promoted osteogenic differentiation. Analysis by micro-CT, immunohistochemistry, and real-time PCR suggest that BMP-2 induced chondrocytes to deposit minerals, synthesize VEGF, and express the mineralization-associated protein osteocalcin.

DISCUSSION: Taken together, our data shows that UCB plays a significant role during normal bone and cartilage growth and development, and that mutations in UCB can result in bone and cartilage malformation. Furthermore, UCB protein may be a promising tool for promoting cartilage regeneration.

APPROXIMATING THE EXTRACELLULAR MATRIX OF THE CORNEA BY ELECTROSPINNING COLLAGEN SCAFFOLDS

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INTRODUCTION. In this project, we seek to recreate the corneal microenvironment as part of a larger effort to create a tissue-engineered cornea. The cornea is composed of cells and organized nano-sized collagen type I fibers. A protocol was developed for electrospinning nano-sized fibers that would replicate the arrangement of collagen in the natural cornea. The goals of this project were to create aligned, 30nm diameter collagen fibers, prepare them for cell culture, and to observe and quantify the effect of the scaffold microstructure on the protein expression of human corneal keratocytes.

METHODS. Aligned type I collagen fibers were prepared using the dual plate electrospinning method. In addition, fiber diameter was controlled by manipulation of solution concentration and viscosity. The resulting scaffolds were prepared for cell culture by developing a method for crosslinking the fibers so that they would not dissolve in cell culture media. SEM and immunofluorescence microscopy were used to quantify changes in cell density and protein expression as a result of the engineered scaffold.

RESULTS. We were able to electrospin 30nm diameter aligned collagen scaffolds and crosslink them for cell culture. Cells grown on aligned mats became elongated along the axis of the fibers, whereas cells on unaligned small diameter scaffolds and cells on culture dishes did not. We also looked for expression of α -SMA, an intracellular protein expressed in the wound healing phenotype of corneal keratocytes but not expressed in a normal transparent cornea. This protein was expressed significantly less in cells grown on aligned scaffolds, indicating that the cells are behaving more like normal keratocytes when presented with a microenvironment similar to that found in vivo.

DISCUSSION. Our results suggest that the aligned cross-linked collagen fibers provide a promising scaffolding material for engineering artificial corneal tissue. Decreased expression of α -SMA in cells cultured on aligned fibers suggests that this scaffolding material may promote better transparency in our tissue engineered constructs.

REGENERATION OF ADRENOCORTICAL TISSUE WITH NILE RED^{DIM} CELLS

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INTRODUCTION: Nile red, a fluorescent hydrophobic probe, is a phenoxazone dye that intensely fluoresces in hydrophobic lipids such as cholesteryl ester. Nile Red can be utilized to develop a sensitive fluorescent stain for tissue lipids and for fluorescent activated cell sorting. In this study, we demonstrated that the adrenal gland can be characterized by Nile red and can be separated into different groups of cells according to Nile red staining.

METHODS: Murine adrenal cells were harvested and stained with Nile Red. Stained cells were sorted into the bright and the dim population. Sorted cells were then analyzed for purity. These cells were then seeded on a collagen sponge. When attached, the constructs were implanted under the renal capsule of the recipient mice that were unilaterally adrenalectomized. These implants were retrieved at 10, 28 and 56 day. The expression of Sf1, Dax1, Cyp11b1, and Cyp11b2 were analyzed by qRT-PCR.

RESULTS: Stained cells were separated into the two distinct groups according to Nile red fluorescence. The purity was confirmed by the post sort analysis. The collagen sponges seeded with sorted cells were implanted under the renal capsule without complication. The retrieved implants showed vessel formation at as early as 10 days. Adrenocortical genes were detected at significant levels on the bright and the dim groups at day 10. After 28 days of implantation, the expression of adrenocortical genes was at a minimally detectable level for both groups. At 56 days, the constructs seeded with the Nile-red-dim population showed significantly higher levels of adrenocortical genes as compared to the Nile-red-bright group.

DISCUSSION: Sorted cells showed distinct features in the Nile red dim and bright groups. The cells in the Nile-red-dim group expressed Sf1, a developmental marker, but none of the functional marker, such as Cyp11b1 and Cyp11b2. At the same time, the cells in the Nile-red-bright group expressed Sf1, Cyp11b1 and Cyp11b2. This indicates a separation of the progenitor cells from the differentiated adrenocortical cells. The Nile-red-dim group further showed the expression of functional markers at the 56th day. The Nile-red-bright group, on the other hand, lost the expression of all adrenal markers, suggesting that the cells from the Nile-red-bright group did not support the regeneration of the adrenocortical tissue.

(SESSION ONE)

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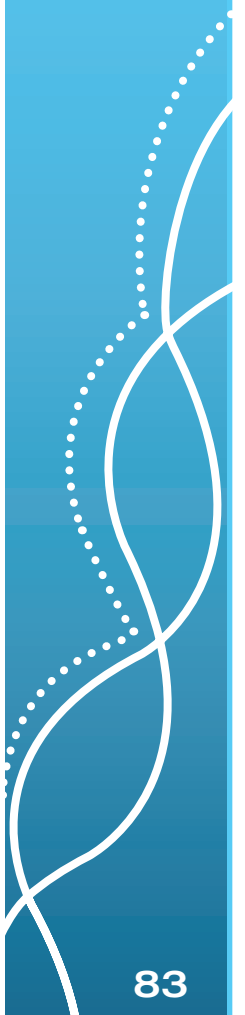
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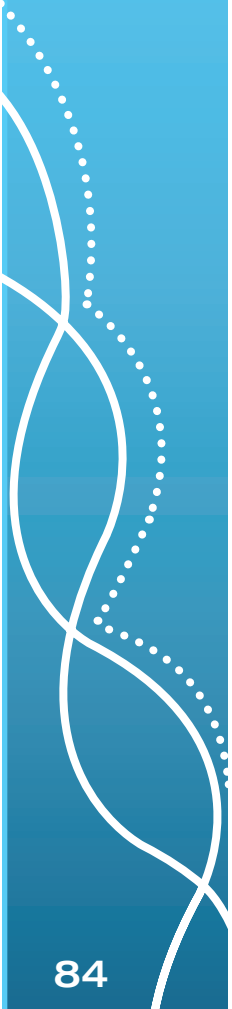
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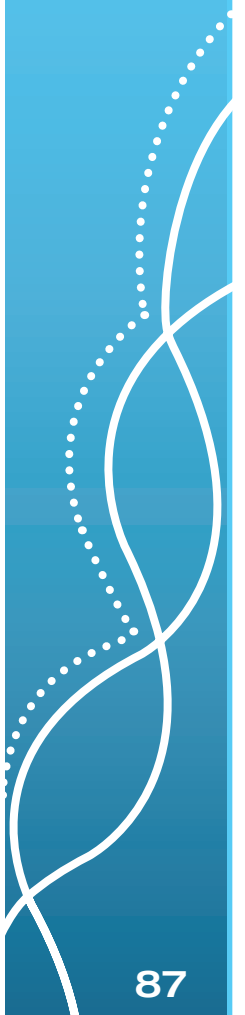
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