



Los Angeles Tissue Engineering Initiative Second Annual Meeting

Saturday, December 4, 2004
California Lutheran University

Sponsored by:

Baxter BioScience

Center for Integrated Science and Bioengineering

California Lutheran University

December 4, 2004
8:00 - 4:00

Los Angeles Tissue Engineering Initiative



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Warren Garner
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Mike Shaw
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Dennis Revie
CLU

Bill Tawil
Baxter BioScience

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Program Design
Martha Rich
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Welcome Letter

Dear Colleagues and Friends:

It is our pleasure to welcome you to the Second Annual Meeting of the Los Angeles Tissue Engineering Initiative (LATEI), held December 4, 2004, on the CLU campus. The goal of LATEI is to provide an interdisciplinary scientific venue for students and investigators in the greater Los Angeles region to meet and exchange knowledge in tissue engineering and related fields. LATEI is open to all interested individuals, with an emphasis of providing graduate and undergraduate students the opportunity to participate in technical discussions with academic and industrial scientists and investigators. A specific goal of LATEI is to foster new interactions between investigators from traditionally disparate scientific disciplines.

The fall LATEI Annual Meeting is held in partnership with the spring Los Angeles Wound Healing Meeting at USC. These Los Angeles-based meetings further complement the California Tissue Engineering Meetings, the most recent of which was held at Stanford University, in October, 2004. With your involvement, we will continue to develop these opportunities for increased communication and collaboration between students, faculty and researchers.

The Second Annual Meeting of LATEI consists of four technical sessions: the first on Soft Tissue Repair; the second on Cardiovascular Tissue Engineering, the third on Bone and Cartilage Tissue Engineering and the fourth on Stem Cell Tissue Engineering. Each session will include opportunities for questions in addition to ample time outside the sessions for informal discussions and interactions. All participant meeting costs are covered by CLU's program in Bioengineering and by generous support from Baxter BioScience.

Thank you for joining us at this event and we are looking forward to a productive meeting!

LATEI Organizing Committee

Los Angeles Tissue Engineering Initiative

Program Schedule

- 8:00 - 8:30 Registration and Continental Breakfast
- 8:30 - 8:40 Welcome & Opening Remarks: *Mike Shaw*, Director, Bioengineering, CLU
- Podium Session 1: Soft Tissue Repair.**
Moderators: *Tai-Lan Tuan*, Children's Hospital & *Mike Shaw*, CLU
- 8:40 - 9:00 Clinical Corner:
Applications of Plastic Surgery to Tissue Engineering.
Kouris Azar, Los Robles Medical Center
- 9:00 - 9:20 LATEI Team-1:
The Mechanical Characteristics of Fibrin – Fibroblast and Fibrin-Collagen Constructs.
Cesar Costales, LATEI Team - 1: CLU, USC, UCLA, Baxter BioScience
- 9:20 - 9:40 Cell Therapy Corner:
The Application of Amniotic Fluid Stem Cells for Renal Tissue Regeneration.
Roger De Filippo, Children's Hospital
- 9:40 - 10:00 Industry Corner:
Development of a Novel Composite Skin Substitute for Clinical and Research Application.
Habib Torfi, InVitrX
- 10:00 - 10:30 Coffee Break
- Podium Session II: Cardiovascular Tissue Engineering.**
Moderators: *Dennis Revie*, CLU, *Bill Tawil*, Baxter BioScience
- 10:30 - 10:50 Novel Technology Corner:
MEMS & Nano Sensors to Predict Cardiovascular Events.
T. K. Hsiai, USC
- 10:50 - 11:10 Student Corner:
Oxygen Diffusion in Engineered Heart Tissue
David A. Brown, UCLA
- 11:10 – 11:30 In Situ Cardiac Tissue Engineering for Myocardial Repair.
Randy Lee, UCSF
- 11:30 - 1:00 Lunch (provided): Overton Hall
- 11:30 - 1:00 LATEI Committee Meeting

Los Angeles Tissue Engineering Initiative

Program Schedule

Podium Session II: Cardiovascular Tissue Engineering Continued.

Moderators: *Dennis Revie, CLU, Bill Tawil, Baxter BioScience*

1:00 - 1:20 Progress in Developing a Composite, Tissue-Engineered Aortic Valve.
Ivan Vesely, Children's Hospital

1:20 - 1:40 Industry Corner:
Sheet-Based Tissue Engineering.
Nicolas L'Heureux, Cytograft

1:40 - 2:00 Coffee Break

Podium Session III: Bone & Cartilage Tissue Engineering.

Moderator: *Geoff Dougherty, CSUCI*

2:00 - 2:20 Student Corner:
The Role of Nell-1 in Bone Formation, Fusion, and Regeneration.
Katie Cowan, UCLA

2:20 - 2:40 Mechanics of Biological and Hybrid Material Systems.
Sridhar Narayanaswamy, Rockwell Scientific Company

Podium Session IV: Stem Cell Tissue Engineering.

Moderator: *Dan Farkas, Cedars-Sinai Medical Center*

2:40 - 3:00 Osteogenic Potential of PLA Derived Adult Stem Cells.
Sanjay Dhar, UCI

3:00 - 3:20 Signal Transduction Pathways Involved in ASC Osteogenic Commitment and Differentiation.
Patricia Zuk, UCLA

3:30 - 4:00 Closing Remarks and Reception
Ben Wu, UCLA

Please be sure to pick up your LATEI T-shirt.

**See you next year at the Third Annual
Los Angeles Tissue Engineering Meeting on Saturday December 3rd, 2005**

Biographies

Kouris Azar

received his B.S. in Biomedical and Electrical Engineering in May 1990, and his M.D. in May 1995 from the School of Medicine, both from the Case Western Reserve University in Cleveland, Ohio. He performed a Fellowship in Plastic and Reconstructive Surgery, between July 1, 2000 to June 30, 2002 at the University of Utah and his General Surgery Residency, July 1, 1995 to June 30, 2000 at the University of Arizona, in Tucson, Arizona.

David A. Brown

is a Ph.D. candidate in biomedical engineering at UCLA. His research focuses on regenerative therapies for the treatment of heart disease by two approaches: construction of tissue-engineered cardiac grafts; and in situ regeneration of diseased myocardium. Tissue engineering of heart muscle on a clinical scale is contingent on a robust and efficient transport system which will mimic many properties of the heart vasculature. In efforts to establish engineering guidelines for providing adequate nutrient transport in tissue-engineered heart muscle, Dave's dissertation work focuses on the cellular effects of oxygen diffusion in an engineered heart tissue model consisting of neonatal cardiomyocyte suspended in collagen gels. He is also working in collaboration with the Department of Medicine on a delivery system of stem cell homing factors to the diseased heart, in which it may be possible to enhance the recruitment of cells aiding in the stabilization or regeneration of diseased myocardium. His thesis advisor is Professor Ben Wu.

Dave graduated from the University of Colorado at Boulder in 1999 with a degree in aerospace engineering and a minor in astrophysics. During his last years as an undergraduate, his interest in engineering drifted towards biological systems; in large part due to his involvement in research projects concerning the physiological effects of spaceflight and the effects of simulated microgravity on antibiotic production.

After seven months of teaching English and traveling throughout Asia, Dave began the graduate program in biomedical engineering at UCLA. During the summer of his first year of graduate school, Dave spent three months working in a rural medical clinic in Guatemala, where he became convinced of his decision to improve public health through both research and clinical work. Dave has been accepted to the UCI College of Medicine, where he will begin his medical training in the fall of next year. He has worked as a teaching assistant in physics for over four years, as well as staying active in the UCLA chapter of Engineers Without Borders. Dave is an avid mountain climber, triathlete, and aspiring Corvette mechanic.

Catherine Cowan

is currently a Ph.D. student in the Department of Bioengineering at the University of California, Los Angeles. Co-mentored by Professor Ben Wu and Professor Kang Ting, she is investigating the role of Nell-1 in bone regeneration in critical sized rat calvarial defects. Interests focus on the Nell-1 signaling pathway, Nell-1 delivery *in vivo*, pharmacokinetics, and Nell-1's role in bone regeneration. The ultimate goal of this research is to advance bone regeneration therapies.

Catherine Cowan received her B.S. degree in Cellular, Molecular, and Developmental Biology in 1997 at the University of California, Santa Barbara, and her M.A. degree in Biology in 1999 at California State University, Dominguez Hills. Her M.A. research was conducted at the Harbor-UCLA Medical Center, and focused on the Alpha-fetoprotein and Retinoic Acid and their roles in apoptosis of hepatoma cells.

Upon graduation, she moved to Palo Alto and began working for Stanford University in the Division of Infectious Diseases. This work consisted of virology and molecular biology studies on the Cytomegalo Virus (CMV), investigating strain differences and patient resistance.

Biographies

After one year, she relocated to the laboratory of Dr. Michael Longaker in the Department of Surgery at Stanford University. Here she investigated the differences in bone reossification between immature and mature animals. Research projects included investigating the differences between juvenile and adult derived osteoblasts (*Journal of Biological Chemistry* 2003) and bone tissue engineering using bioactive scaffolds from Dr. Wu's lab (*Nature Biotechnology* 2004, *Tissue Engineering* 2004).

Roger De Filippo

is Assistant Professor, Division of Urology at Children's Hospital Los Angeles. He received his B.S. with Honors in the Biological Sciences from the University Southern California, and his M.D. from the University of Southern California School of Medicine, where he finished his Urology Residency as well. Recently he finished a Fellowship in Pediatric Urology at Children's Hospital in Boston. In 2000, He was awarded the Von L. Meyer Traveling Fellowship and was the first place recipient for Basic Science Research in Tissue Engineering from Children's Hospital in Boston.

Sanjay Dhar

As Director of the Laboratory of Tissue Engineering and Regenerative Medicine at the University of California, Dr. Sanjay Dhar has an extensive research history studying the effects of different cytokines on varying target cells. Dr. Dhar completed his graduate studies at the Indian Veterinary Research Institute in Molecular Parasitology, and his Post-doctoral work was conducted at the New England Medical Center of Boston.

T.K. Hsiai

received his undergraduate education from Columbia University, and MD from the University of Chicago. Dr. Hsiai completed his cardiology fellowship and PhD in Biomedical Engineering at UCLA School of Medicine in 2001. He is currently professor of biomedical engineering and cardiovascular medicine at USC. Dr. Hsiai is a Fellow of American

College of Cardiology and a recipient of NIH Physician Scientist Career Development Award.

Randall Lee

is Associate Professor of Medicine at the University of California, San Francisco, and the Director of the Bioengineering Graduate Group, which is a combined program between University of California Berkeley and San Francisco campuses. He completed his Medical degree and Doctoral degree in Pharmacology from the University of California. Dr. Lee has recently been researching different techniques of improving tissue engineering of cardiac tissue.

Sridhar Narayanaswamy

is a research Scientist in the composite materials group at Rockwell Scientific. He obtained his B. Tech. degree (Metallurgy, 91) from India, MS (Materials Science), MS (Mechanical Engineering) and Ph.D. (Materials Science) degrees, all from the University of Michigan, Ann Arbor. Dr. Narayanaswamy's primary research areas are in the mechanics of structural composites, electronic materials, fracture mechanics, and computational materials science. He has authored or coauthored 35 research publications.

Habib Torfi

President and Chief Science Officer. He has 15 years experience in development of 3-D tissue engineering models. He has numerous publications and a BS in Microbiology and Human Genetics from Weber State University in Utah.

Ivan Vesely

is the Director of the Cardiovascular Research Program at Children's Hospital in downtown, Los Angeles. He attended the University of Western Ontario. His studies are designed to investigate the biomechanical effects of "bending" on tissue. He is presently working on the Micromechanics of the Aortic Valve, Composite Tissue Engineered Aortic Valve Prosthesis, and Advanced Soft Tissue modeling for Telemedicine and Surgical Simulation.

Applications of Plastic Surgery to Tissue Engineering.

Kouris Azar, Los Robles Medical Center

Tissue engineering is an interdisciplinary field that applies the principles of engineering and life sciences to the development of biologic substitutes to restore, maintain, or improve tissue function. Tissue engineering has opened new avenues for plastic surgeons to reconstruct tissue defects using selective cell transplantation instead of whole tissue or organ transplantation. These approaches have several obvious advantages for tissue reconstruction. First, functional tissue theoretically could be generated in vitro or in vivo using cells from a small amount of donor tissue by expanding them in vitro to create a potentially limitless supply. Second, the ability to induce differentiation of isolated cells could enable us to engineer functional tissue using cells from different origins. There are many aspects of reconstructive surgery from facial and ear reconstruction to chest wall and abdominal reconstruction after cancer and trauma that will benefit in the near future from advances in tissue engineering. We will review a variety of reconstructive surgery challenges with actual patient examples looking for applications and frontiers of tissue engineering in surgery.

Indentation Micromechanics of Fibroblast-Fibrin and Keratinocyte- Fibrin Constructs.

C. Costales, R.G. Mooney, A. Doerfler, G. Toland, W. Garner,¹ B. Tawil,² T.-L. Tuan,³ B. Wu⁴ and M.C. Shaw, Bioengineering Department, California Lutheran University, 60 W. Olsen Rd. #3750, Thousand Oaks, CA 91360,¹Section of Plastic Surgery, Keck School of Medicine, University of Southern California, 1200 N. State St., Rm 12650, Los Angeles, CA 90033, ² Baxter Healthcare Corporation, One Baxter Way, Westlake Village, CA 91362, ³ Keck School of Medicine, 1510 San Pablo, HCC 514, University of Southern California, Los Angeles, CA 90089-9202, ⁴Department of Biomedical Engineering, University of California, Los Angeles, California 90095

Introduction:

Skin is the largest organ in the body and forms a critical protective barrier to the external environment [1]. During normal wound healing, fibrin clots form within the first few minutes and transform into dermal and epidermal tissues over several days. To assist this process, recently developed fibrin-based sealants, consisting of two primary components, fibrinogen and thrombin which form a gelatinous clot when mixed, have been studied in vitro and in animal studies for cell and bioactive substance delivery [2 – 7]. However, the dependence of the structural mechanics of the clot on its biochemistry and cellular response is not yet known. In the present investigation, a well-established indentation protocol [e.g., 8] was applied to determine the time-dependent and viscoelastic parameters of the cell-fibrin constructs, and effects of clot biochemistry and cellular response.

Materials and Methods:

Fibrinogen – containing and Thrombin Solution Preparation/Cell Culture: Fibrinogen – containing and thrombin solutions were diluted to the appropriate concentrations by diluting fibrinogen – containing solution in TBS and Thrombin in 30mM CaCl₂ in TBS. Two cell lines were purchased from ATCC: 1. Human fibroblast cell line CRL-2522: propagated using MEM and 10% fetal bovine serum. 2. Human keratinocyte cell line: CRL-2404: propagated using Keratinocyte-Serum Free Medium with human recombinant EGF and L-glutamine. Following culture, T-75 flasks containing cells were rinsed twice with 10 mL pre-warmed serum-free medium, once with 6 mL pre-warmed Trypsin and then were incubated with 6 mL pre-warmed Trypsin in a 5 % CO₂ incubator at 37°C until the cells start to detach. After the cells detach, 12 mL of Trypsin Neutralizing Solution is added to the flasks and the cell suspension is transferred to a 50 mL centrifuge tube. The flasks were rinsed with 10 mL of a serum – free medium and the rinse is added to the centrifuge tube. The tube is centrifuged at about 2000 rpm for 6 minutes and the supernatant is discarded. The cell pellet is washed with 5 mL of a serum-free medium, centrifuged at about 2000 rpm for 6 minutes and resuspended in 1 mL of pre-warmed TBS. Cells were counted using a standard Hemacytometer. Cells were mixed with the reconstituted Fibrinogen - containing solution and then diluted using TBS. The number of cells added was 334,000 cells per mL of Fibrinogen – containing solution or 50,000 cells per fibrin clot.

Indentation Micromechanics of Fibroblast-Fibrin and Keratinocyte- Fibrin Constructs, Continued

Cell – Clot Constructs Preparation: 400 μ L – 10,000 mL of the Fibrinogen – containing solution with cells was added to each well of 6, 12 or 24 well plates. Two plates were prepared for the following time points Day 0, 1, 5, 10 and 15. 150 μ L Thrombin solution was added and the wells were mixed well by tapping and tilting the plates. The plates were incubated at room temperature for 2 hours. Fibrin clots were washed twice with 1 mL of a warm serum-free medium. 1 mL of a serum – containing medium was added to each well and plates were transferred to a 5% CO₂ incubator at 37°C. The medium was replaced on Days 1, 3, 5, 7, 10 and 12 with 2 mL of a serum-containing medium.

Indentation Assay: The fibrin/cell constructs were subject to a compressive indentation protocol wherein a circular, flat-ended glass indenter was applied to the surface of the cell/fibrin constructs through controlled displacements until reaching a specified maximum displacement, u^* . An Instron 3365 Universal Testing Machine equipped with a 2.5N load cell with a load resolution of 0.1 mN was used. Displacement rates in the range of 0.1 –10 mm/min were used, with peak displacements, $u^* = 0.1 -5$ mm. The force required to maintain these displacements was automatically recorded.

Results and Discussion:

Initial results reveal significant differences in the linearized Young's modulus obtained from the indentation experiments for different formulations of populated and unpopulated fibrin and collagen construct formulations. These results demonstrate the applicability of the indentation technique to assay the mechanical properties of cell-containing fibrin-collagen constructs *in vitro*.

References:

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- 2) Tuan, T. L., Song, A., Chang, S., Younai, S., and Nimni, M. E. Experimental Cell Research **223**, 127, 1996.
- 3) Wechselberger, G., Schoeller, T., Stenzl, A., Ninkovic, M., Lille, S., Russell, R. C, The Journal of Urology **160**, 583, 1998.
- 4) Horch, R. E., Bannasch, H., Kopp, J., Anree, C., and Stark, G. B. Cell Transplantation **7**, 309, 1998.
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- 8) Lawn, B, "Fracture of Brittle Solids," Cambridge University Press, 1993.

The Application of Amniotic Fluid Stem Cells for Renal Tissue Regeneration.

L. Perin, A. Maeshima, Sedrakyan S., Jin D., SK. Nigam, A. Atala and R. De Filippo

The creation of bio-artificial organs like the kidney by Tissue Engineers showed promise but significantly falls short of reproducing all of its multifaceted structure and function. Historically, tissue engineers were dependent entirely on adult cell lines that resulted in simple two dimensional constructs that exhibited limited function and were difficult to apply in vivo. Stem cell based therapies may help overcome some of these difficulties. A pluripotential stem cell population exists in amniotic fluid (AFSC). These cells have been shown to differentiate into all three germ layers and participate in the formation of various tissues during embryogenesis. Complex organs like kidney are composed of two major tissues: epithelium and mesenchyme. We applied a novel system of tissue regeneration from AFSC to achieve renal cell differentiation. A co-culture system was employed using essential constituents of developing kidney: the ureteric bud (UB) and metanephric mesenchyme (MM). We hypothesized that differentiation of AFSC into renal cells can be achieved by induction of growth factors released during the culture of UB and MM in vitro. We determined the capacity of embryonic UB and MM to induce AFSC differentiation in a novel co-culture system that allows for interchange of growth factors. Induction of AFSC into renal tissue was confirmed morphologically and histologically.

Development of a Novel Composite Skin Substitute for Clinical and Research Application.

R.K. Kordestani¹, E. Elmore², J.S. Steinberg³, P. Radovic⁴ and H. Torfi¹.

Introduction:

Chronic non-healing wounds and burns continue to be a challenging problem. Xenografts of composite skin substitutes have been used for therapeutic interventions. Due to the inherent problems with xenografts, no ideal skin substitute yet exists. Invitra™ is a novel Composite Skin substitute (CSS) that is grown from a neonatal skin cells and offers a dermal components in a 3-dimensional structure. Allogeneous fibroblasts are isolated, cultured and then are placed in a biodegradable proprietary matrix. To prepare Invitra™, a small piece of the neonatal skin is enzymatically digested using standard techniques. Following culture expansion, fibroblasts are mixed in modified Vitrogen (Cohesion Technologies, Palo Alto, CA) and layered onto a biodegradable matrix consisting of Vicryl mesh (Ethicon Biosurgery, Cincinnati, Ohio) and Surgisis (Cook Biotech Inc. Indiana), and allowed to gel. These grafts are ready for delivery and or grafting after two days. The resulting 3-dimensional construct is ready for use.

Materials/Methods:

A 1-cm² biopsy of human skin containing both dermal and epidermal components is harvested^{3,4}. The skin is enzymatically digested, yielding keratinocytes and fibroblasts, which are separated using standard techniques⁵. The cells are then incubated in splitting solution for 16-21 hours at 4°C. Once incubated, the dermis and epidermis are teased apart. The epidermal pieces are incubated in rTE solution (Cascade Biologic, Inc. Portland, OR) for 30 minutes in a 37°C water bath. Cells are resuspended in Epilife Medium supplemented with HKGS-V2 and PSA (Cascade Biologic, Inc.). Fibroblasts cells are mixed at 5.0x10⁵ cell/ml in Vitrogen and poured unto the biodegradable matrix composed of two parts, Vicryl mesh (Ethicon Biosurgery, Cincinnati, Ohio) and Surgisis (Cook Biotech Inc. Indiana), and allowed to gel.

Results and Discussion:

A 66-year-old diabetic male with severe peripheral vascular disease developed gangrene of the distal left hallux. He underwent incision and drainage with a partial amputation of the hallux in anticipation of revascularization, which was scheduled later in the week. The revascularization was delayed and gangrene developed proximally. A partial first ray amputation was performed and the wound was partially closed. Revascularization was rescheduled for later that week. In the meantime a wound vacuum assisted closure (wound V.A.C., KCI, San Antonio, Texas) was placed on the foot. The patient was revascularized and once the wound filled in with granulation tissue, the wound V.A.C was discontinued and a hydrofoam dressing was applied until the Invitrx skin graft was ready. Two applications of Invitra - CSS were applied over a two-week period and rapid healing was noted.

Development of a novel Composite Skin Substitute for clinical and Research Application. Continued

References:

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2. Navsaria, H et al. Culturing skin *in vitro* for wound therapy. *Tibtech* 1995; 13:91-99.
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5. Cascade Biologics, Inc. Epilife Medium Supports the Isolation and Extended Cultivation of Human Corneal Epithelium. Cascade Biologics, Portland, OR.
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MEMS and Nano Sensors to Predict Cardiovascular Events.

T.K. Hsiai, USC

Cardiovascular disease remains the leading cause of morbidity and mortality in the United States. Hemodynamics, in particular, shear stress, plays an important role in regulating the biological activities of endothelial cells that line the inner lumen of blood vessels. The emerging Micro Electro Mechanical Systems (MEMS) provide a spatial resolution comparable to the size of EC and temporal resolution in the kHz range to investigate the mechanisms whereby the characteristics of shear stress regulate the biological activities of endothelial cells. Using tools originally developed for the integrated circuit (IC) industry, one is able to fabricate miniaturized transducers at the cellular levels for real-time control of time-varying events.

In parallel, the study of nanomaterials and nanoscale science paves a pathway for an ambitious but realistic goal: the use of synthesis, assembly and miniaturization down to the nanometer scale to create novel nanostructured materials and devices with unique properties and superior performance. The development of nanowire sensors provides a new avenue to understand the fundamentals of oxidative modification of low density lipoprotein-cholesterol (LDL) mediated by vascular endothelial cells in response to shear stress. In this context, our group at USC has integrated expertise from the fields of micro- and nanotechnology, vascular and oxidant biology to study the dynamics of inflammatory responses to predict acute coronary events.

Oxygen Diffusion in Engineered Heart Tissue.

David A. Brown, UCLA Bioengineering

Introduction:

Progress in tissue engineering of clinically-relevant cardiac patches is currently hindered by inadequate diffusion of gases and nutrients into the cores of tissue constructs, resulting in a maximum tissue thickness of less than 1 mm. Based on oxygen profiles in tumors and various engineered tissues, it is suspected that oxygen may be the limiting metabolite for viability in many engineered tissue types including myocardium. A model for oxygen diffusion in cardiomyocyte-collagen gel constructs has been developed to more precisely characterize this phenomenon, which will aid in the design of an efficient transport system for engineered heart tissue.

Methods:

Neonatal cardiomyocytes were suspended in a collagen-Matrigel hydrogel at various cellular densities, which was then sandwiched between two circular cover slips; hence limiting diffusion of oxygen from the bulk medium to the radial direction. Gels were then cultured overnight in different concentrations of oxygen and fresh-frozen for histological cryosectioning.

Results:

Radial gradients of oxygen were observed by immunohistochemical staining for pimonidazole, which conjugates to cellular proteins in the absence of oxygen ($<2\% \text{O}_2$). Pimonidazole staining was clearly visible around the gel section in all O_2 conditions, indicating a rapid depletion of oxygen in the tissue construct. Gels cultured in $0\% \text{O}_2$ showed maximum pimonidazole staining intensity at the edge of the gel as expected, while those cultured in $21\% \text{O}_2$ and $70\% \text{O}_2$ stained maximally deeper into the gel. Severe cell death was observed in the interior of the gel, with larger proportions of TUNEL-positive cells visible in the interior with decreasing O_2 . Increasing cell density had an analogous effect to decreasing O_2 . These data will be presented with a diffusion model based on anisotropic oxygen diffusivity in an effective medium with Michaelis-Menten-type consumption.

Conclusions:

Concurrent with reports of other investigators, the maximum length scale for normoxic conditions in engineered heart tissue is on the $100\text{-}\mu\text{m}$ scale under diffusion-restricted culture. Cardiomyocyte hypoxia and death in collagen gels are functions of cell density and head gas oxygen concentration, which can be predicted through oxygen diffusion models. Ongoing work focuses on further characterization of transport-limited cell viability and function for cardiac tissue engineering.

In Situ Cardiac Tissue Engineering for Myocardial Repair.

Karen L. Christman, Qizhi Fang, Ngan F. Huang, Michael Yee, Richard E. Sievers, *Randall J. Lee*.
University of California San Francisco, San Francisco, CA, 94143

Myocardial infarction (MI) resulting in the loss of non-regenerative cardiomyocytes, negative remodeling of left ventricle (LV) and the deterioration of myocardial function is still a major cause of congestive heart failure.^{1,2} The use of biopolymers is an emerging area of research in cardiac tissue engineering and shows tremendous promise in myocardial repair. As an injectable scaffold, fibrin results in improved cell survival and angiogenesis.³ Additionally, fibrin matrix alone has been shown to decrease infarct expansion and prevent the negative remodeling associated with an acute myocardial infarction.^{3,4}

Recently, we examined the effect of this in situ approach in a chronic MI model. The left coronary artery of Sprague-Dawley rats was occluded for 30 minutes followed by reperfusion. A baseline echocardiogram was performed by a blinded investigator five weeks post-infarction on all animals. Either 0.5% bovine serum albumin (BSA) in PBS (control) (n=7), 2×10^6 skeletal myoblasts (SM) in 0.5% BSA (n=6), FG alone (n=8), or 2×10^6 SM in FG (n=6) was then injected into the ischemic left ventricle (LV). A follow-up echocardiogram was also performed five weeks after injection. Paired t-tests were used to compare measurements between baseline and follow-up echocardiograms in order to determine the effect of each treatment. As anticipated the BSA control group exhibited a significant decrease in ejection fraction (a measure of cardiac function) ($p < 0.004$), a significant increase in LV diameter ($p < 0.01$), and a significant decrease in infarct wall thickness ($p < 0.01$). Furthermore, the SM in BSA group was also incapable of preserving cardiac function and LV geometry. In contrast, both injection of FG and SM in FG preserved cardiac function and LV geometry five weeks after injection.

The results of these studies indicate injection of a fibrin scaffold helps to maintain LV geometry and cardiac function, and thus may be a potential treatment for those suffering from chronic myocardial function.

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Progress in Developing a Composite, Tissue-Engineered Aortic Valve.

Yaling Shi, Ivan Vesely, Saban Research Institute, Childrens Hospital Los Angeles, Los Angeles, California 90027, USA

Introduction:

A tissue-engineered valve must include the complex microstructure of native valves in order to mimic their unique mechanical properties. The functional components include collagen fiber bundles, mesh networks, branches, and layers of elastin. Our approach is to engineer each structural component of the heart valve separately in vitro and then integrate all the components together into a composite valve structure. Recent progress has been directed towards growing branched and connected collagen fiber bundles and elastin sheets, and towards optimizing the culture conditions and the bioreactors involved, to continually improve the mechanical strength of the constructs.

Methods:

Collagen fiber bundles were fabricated using directed collagen gel shrinkage. Neonatal rat aortic smooth muscle cells were mixed with acid-solubilized type I rat-tail collagen and gelled within silicon rubber wells of variable geometry. Single-branched constructs as well as more complex geometries were developed, and static and dynamic loading protocols were explored during 8 weeks of culture. Additional collagen constructs were cultured separately for 4 weeks under static tension, and then placed in direct contact with each other and cultured for an additional 4 weeks to investigate how well they integrate together. Culture medium optimization was carried out by varying the nutrient makeup of test cultures and assaying for cell and collagen content after 4 weeks of culture. Bioreactor design involved the evaluation of numerous holder materials and geometries, and the use of constant strain or constant tension culture systems. Standard biochemical, histological, and microscopic techniques were used to analyze the constructs. All constructs were also tested mechanically to evaluate the effects of geometry and culture conditions on mechanical strength. Elastin sheets were grown by culturing cells on cross-linked hyaluronan substrates, texturized by UV light irradiation to enhance cells attachment and matrix synthesis.

Results and Conclusions:

The degree of compaction, and hence mechanical strength, varied with mold geometry and other culture parameters. Static and dynamic loading produced stronger constructs than did culture under constant strain. The use of polyester mesh produced greater collagen compaction and stronger constructs that did the glass fiber mesh used originally. Although multibranched constructs had acceptable strength, single, one-dimensional constructs were the strongest. Medium fortified with trace elements and vitamins enhanced cell proliferation and collagen synthesis by 200% and 300% respectively. Exhaustive optimization of culture conditions therefore has a positive effect on the development of tissue-engineered constructs.

Sheet-Based Tissue Engineering

Nicolas L'Heureux, Cytograft

Sheet-based Tissue Engineering (SBTE) is a method in which cells are cultured in conditions that promote extracellular matrix deposition until a sheet, comprised of living cells and a complex endogenous extracellular matrix, is produced. These sheets can be detached from the culture substrate and layered into various geometries to produce a three-dimensional tissue or organ. This approach marks a drastic departure from classic tissue engineering doctrine as it allows the production of autologous living tissues with superior mechanical properties without the need for any exogenous scaffolding.

The clinical relevance of this technology has been recently confirmed by producing Tissue Engineered Blood Vessel (TEBV) from age & risk-matched human cells, i.e. from older patients undergoing vascular reconstruction procedures. These vessels displayed burst pressures twice that of a saphenous vein as well as near physiological compliance and suture retention strength. These age & risk-matched human TEBVs were grafted in athymic rats for up to 250 days and displayed remarkable tissue integration and remodeling. In primates, TEBV diameters were closely followed for 8 weeks to demonstrate mechanical integrity during the critical phase of tissue integration. Future vascular applications of this new platform technology include the production of vascular patches and covered stents.

Role of NELL-1 in Bone Formation, Fusion, and Regeneration.

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Introduction:

Nel-like, type 1 molecule (Nell-1) is a secretory molecule involved in bone formation, fusion, and regeneration. Nell-1 was first discovered as being over-expressed during premature cranial suture closure in human craniosynostosis. Nell-1 signals downstream of Cbfa1 and sufficient to induce osteoblast differentiation and bone formation. Transgenic mice over-expressing Nell-1 display suture overgrowth, while Nell-1 treatment of normal calvarial sutures induces suture overgrowth. In culture, Nell-1 specifically accelerates alkaline phosphatase expression and bone nodule mineralization of osteoblasts. Interestingly, excessive Nell-1 stimulation leads to osteoblast apoptosis. The discovery of Nell-1 in craniosynostosis presents a potential for bone growth in other acquired and congenital craniofacial deformities. Clinical problems requiring bone regeneration are diverse and no single regeneration approach will likely resolve all injuries. The current research utilizes biodegradable PLGA scaffolds coated with Nell-1 for accelerated bone regeneration within the calvaria. Many regeneration strategies use BMP-2 for treatment, but with limited success. The use of Nell-1 may overcome some of the problems associated with BMP-2, including it's specificity for osteoblasts.

Methods:

PLGA scaffolds were coated with 200 ng of rNell-1, rhBMP-2, or no growth factor and implanted into 3 mm full-thickness calvarial defects in Sprague-Dawley rats. Rats were sacrificed after 1, 2, or 4 weeks and examined by microCT. Additional rats were live imaged with a microCT (Imtek Inc., Knoxville, TN) on week 2, 4, 8, and 12 for investigation of bone regeneration in individual animals. Ten micron thick paraffin-embedded sections were examined histologically with Masson-Goldner Trichrome stain. Immunohistochemistry for BRDU (Santa Cruz Biotech, Santa Cruz, CA) was used to examine proliferation as previously described.

Results:

MicroCT analysis allowed for quantification of area and volume within the defects over time. Live imaging, high resolution microCT analysis, and histology demonstrated increasing bone formation in individual animals.

Discussion:

Calvarial defect healing is augmented with growth factor implantation. Nell-1 and BMP-2 are both known to induce bone formation *in vivo*; however, the transition from animal studies into clinical studies has been only mildly successful with BMP-2. The ability of Nell-1 to accelerate bone formation may relate to the decrease in the number of proliferative cells within the defect, suggesting that Nell-1 influenced cells out of a proliferative phase and into a differentiated phase. Nell-1's ability to accelerate calvarial bone regeneration, equivalent to BMP-2, may provide an alternative non-BMP-based paradigm for the study of calvarial bone healing and future tissue engineering studies.

Mechanics of Biological and Hybrid Material Systems.

Sridhar Narayanaswamy, Rockwell Scientific, Thousand Oaks, CA

Natural biological materials such as bone and seashell are nanocomposites of protein and mineral with the toughness of protein and the stiffness of mineral. Although these materials are generally complex with many orders of hierarchical structures, at the most fundamental level of the nanoscale, these systems exhibit extraordinary simplicity and elegance. Simple models will be presented to show that the selection of the nanometer length scale plays a key role in biological systems achieving superior material properties. For instance at this length scale, the mineral platelets in bone achieve optimum strength and the spatula structure of Gecko achieves optimum adhesive strength even in the presence of flaws. Furthermore, models will be presented for estimating the stiffness and fracture energy of biocomposites and identify the important parameters responsible for the superior mechanical properties achieved by biological nanostructures.

Osteogenic Potential of PLA Derived Adult Stem Cells.

Sanjay Dhar, Ph.D., UCI

Human processed lipoaspirated (PLA) derived stem cells have been induced into the bone, cartilage, and fat. Compared with cells harvested by bone-marrow aspiration, PLA cells are easier to obtain, have lower donor-site morbidity, and are available in larger numbers which eliminates the need for costly and lengthy tissue-culture expansion. Isolation of single cell populations of these cells has shown that they have at least potential to form osteogenic phenotype using osteogenesis specific media. Our study has shown that the osteogenic differentiation of these cells post induction can be prolonged for an extended period of time. Thus, ADSC's may be an ideal source for further experiments on stem cell biology and regenerative medicine.

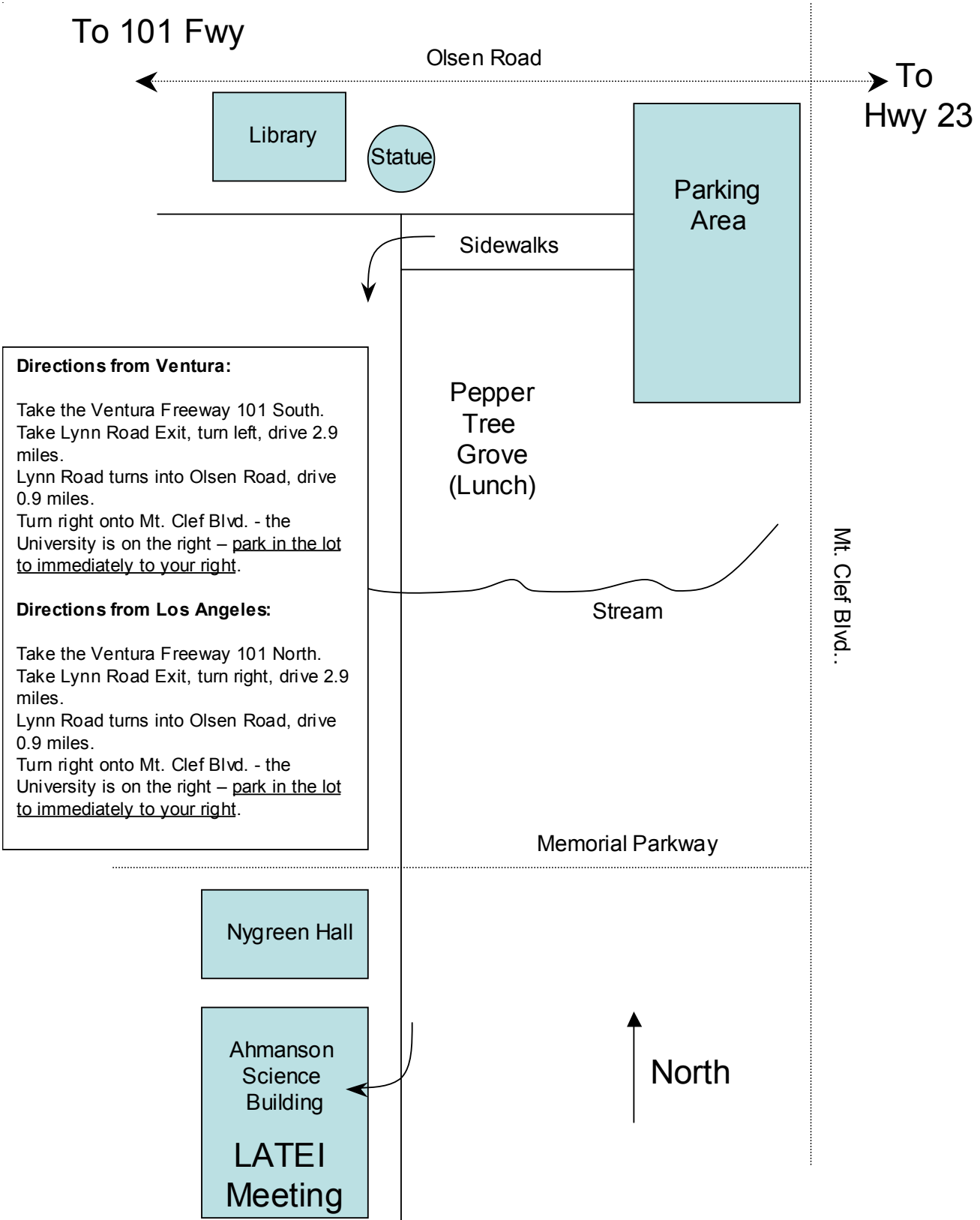
Signal Transduction Pathways Involved in ASC Osteogenic Commitment & Differentiation.

Patricia Zuk, Ph.D., Regenerative Bioengineering & Repair Laboratory; Department of Surgery, David Geffen School of Medicine at UCLA; Los Angeles, CA

Preliminary studies have recently identified a putative stem cell population within the adipose stromal compartment. This cell population, termed **Adipose-derived Stem Cells (ASCs)**, can be isolated from human lipoaspirates and differentiates toward the osteogenic, adipogenic, myogenic and chondrogenic lineages. However, the ASC population, upon harvest and initial culture, is heterogenous. Therefore, to confirm the presence of a stem cell population within adipose tissue, clonal derivatives are necessary. Like the heterogenous population, single ASC-derived clones possess multiple mesodermal potentials, indicative of a multipotent/stem cell capacity. To study the mechanisms behind the clonal ASC osteogenic phenotype differentiation, multiple signal transduction pathways in ASC clones with restricted lineage potentials were examined. To this end, ASC clones with adipogenic, osteogenic and chondrogenic (AOC clones) were compared to clones with in addition to more adipogenic/chondrogenic (AC clone) using microarray and conventional biochemical assays. The tri-lineage AOC clones were found to express multiple signal transduction genes that could be organized into well-established pathways involved in stem cell differentiation. In addition, significant differences in several of the genes of the **MAPK** signaling cascade were measured between the AOC and AC clones using both microarray and real-time PCR analysis, suggesting that this signal transduction pathways plays a role the osteogenic phenotype. Finally, osteogenic induction of the heterogenous ASC population appears to confirm the role of MAPK signaling in osteogenesis.

Los Angeles Tissue Engineering Initiative

CLU Map



Please mark your calendars for these upcoming meetings.

December 2005

We look forward to seeing you next year at these informative meetings.

October 2005



Fourth Annual California Tissue Engineering Meeting

Friday, October 7, 2005
Saturday, October 8, 2005

University of California, Irvine
Irvine, California

Third Annual Los Angeles Tissue Engineering Meeting

Saturday, December 3, 2004

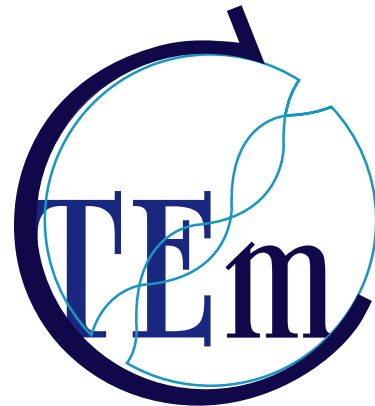
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