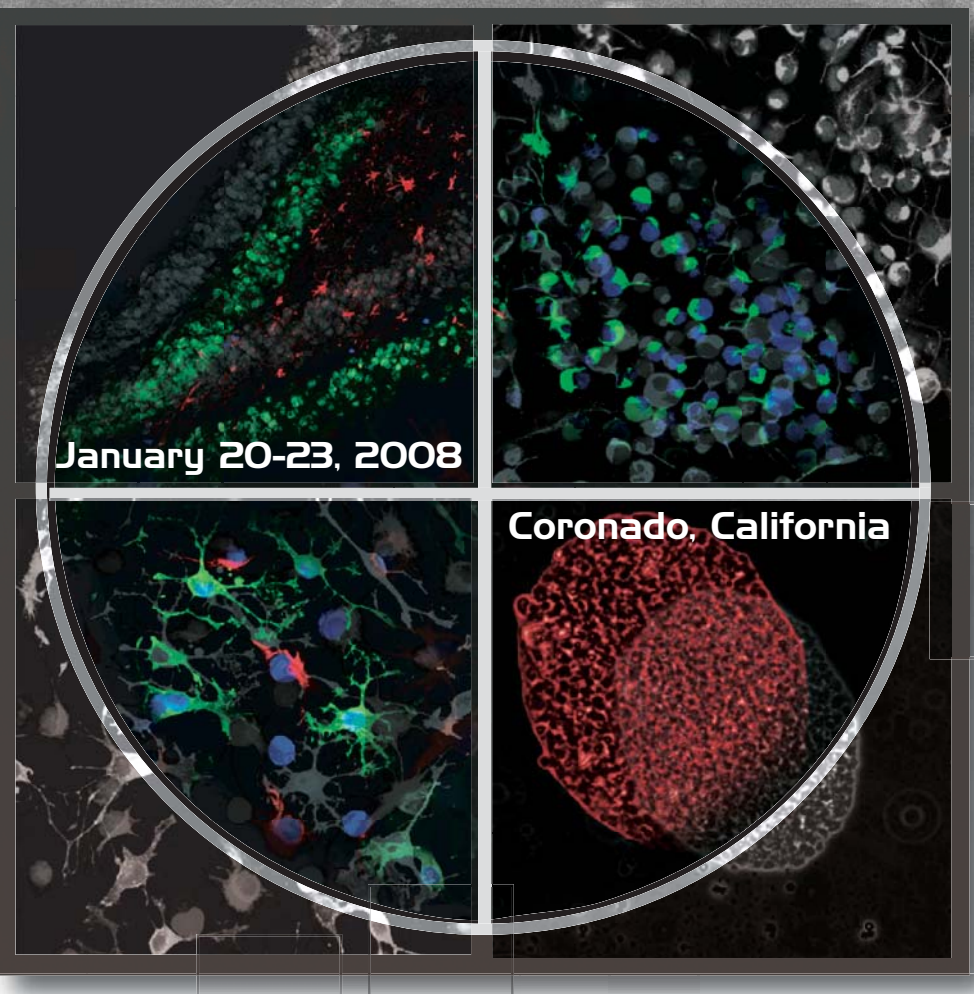
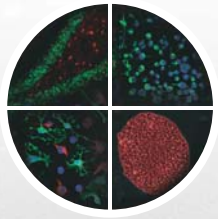


Society for Biological Engineering

SBE's 1st International Conference on Stem Cell Engineering

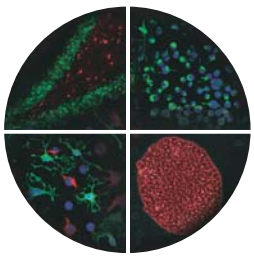
"From Benchtop to Bioprocess"



SBE's 1st International Conference on Stem Cell Engineering

January 20-23, 2008
Coronado Island, CA

Program Book



SBE's 1st International Conference on Stem Cell Engineering

January 20-23, 2008
Coronado Island, CA

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The 2008 International Conference on Stem Cell Engineering is organized by the Society for Biological Engineering

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7:15pm – 8:15pm	**Opening Keynote Address by:** George Daley, *Boston Children's Hospital, Harvard University*	St. Tropez C,D
8:15pm – 10:00pm	**Dinner**	
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8:30am – 10:30am	**Session 1:** High Throughput and Microfluidic Screening Platforms	St. Tropez C,D
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12:30pm – 2:00pm	**Lunch**	La Terrasse
2:00pm – 2:50 pm	**Keynote Address by:** Doug Lauffenburger, *Massachusetts Institute of Technology*	St. Tropez C,D
2:50pm – 4:30pm	**Session 3:** Systems-Based Approaches to Understanding Fate Decisions	St. Tropez C,D
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10:30am – 2:30pm	**Session 5:** Novel Approaches for Embryonic Stem Cell Growth and Differentiation	St. Tropez C,D
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Wednesday, January 23		
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10:30am – 10:50am	**Break**	St. Tropez, Foyer
10:50am – 12:30pm	**Session 8:** Bioreactors and Bioprocesses for Cell Expansion and Differentiation	St. Tropez C,D
12:30pm – 2:00pm	**Lunch**	La Terrasse
2:00pm – 4:00pm	**Session 9:** Biomolecular Scaffolds for Stem Cell Applications	St. Tropez C,D
4:00pm – 4:15pm	**Closing comments by:** David Schaffer, *University of California, Berkeley*	St. Tropez C,D

Greetings!

It's our distinct pleasure to welcome you to Coronado Island and the Society for Biological Engineering's First International Conference on Stem Cell Engineering. Our theme is the "From Benchtop to Bioprocess." We'll be covering the breadth of shaping, and refining our field. There are several areas of research in stem cells that would greatly benefit from an integrated approach that brings together biologists and engineers from academia and industry.

It has become increasingly clear that in their native environment, cells do not respond to individual signals one at a time, but to complex combinations of signals in an intricate fashion. The development of high throughput technologies to vary the identity, combinations, and relative levels of signals and to efficiently analyze the resulting effects on cell function is an important endeavor. Understanding the mechanisms by which cells interpret and respond to complex signals at a systems level requires a combination of biology, engineering, and the physical sciences. In addition, realizing the potential applications of stem cells will require the development of robust cell culture platforms for cell expansion and differentiation. Traditional biochemical engineering approaches to develop defined, scaleable cell culture systems may be important for such endeavors. Finally, translation of stem cells and their differentiated progeny to the clinic will benefit from knowledge gained in the field of tissue engineering, including the development of novel cellular scaffolds engineered for stem cell applications.

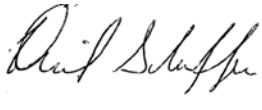
These four areas share a common theme: they will require the intersection of viewpoints, knowledge, and technologies from biology and engineering, from both academia and industry. The time is ripe to bring leading researchers with these complementary backgrounds together to aid in building the field of stem cell engineering, as well as to provide a strong educational forum to train young researchers in this emerging field.

It's against this background and these challenges that we've designed the inaugural International Conference on Stem Cell Engineering. You'll hear from leading researchers with diverse backgrounds who use quantitative approaches to advance the understanding and application of stem cell biology at the molecular, cellular, and tissue level. It is our hope that time shared here this week will stimulate integration of these approaches across the molecular, cellular, and higher scales of biological complexity. The size of the meeting and its format will maximize opportunities for interaction among participants, and we're delighted by the exciting line-up of invited and contributed talks and poster presentations.

We gratefully acknowledge the contributions of colleagues who've made this meeting possible. First, we applaud the Society for Biological Engineering for its role in instituting this conference. We thank our Scientific Advisory Board for its guidance. We also acknowledge the National Science Foundation and the University of California Discovery Opportunity Program, which have supported the participation of many graduate students. Last, but certainly not least, we thank our generous corporate sponsors for the great enthusiasm they've demonstrated for this first Stem Cell Engineering conference.

During the next four days, we hope you'll be inspired by the speakers, actively participate in the poster sessions and receptions, and take some time, as well, to enjoy the beauty of Coronado Island and San Diego.

Thank you for joining us,



David Schaffer
Co-Chair
University of California, Berkeley

Fred Gage
Co-Chair
Salk Institute

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The mission of SBE is to promote the integration of biology with engineering and realize its benefits through bioprocessing, biomedical and biomolecular applications by connecting people, cultivating knowledge, and catalyzing the future.

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Professor Gregory Stephanopoulos, *Massachusetts Institute of Technology*

Sunday, January 20

6:00pm – 7:00pm	**Welcome Reception**	St. Tropez Foyer
7:00pm – 10:00pm	**Dinner**	St. Tropez C, D
7:00pm – 7:15pm	**Introductory Remarks: David Schaffer,** *University of California, Berkeley*	
7:15pm – 8:15pm	**George Daley,** *Boston Children's Hospital, Harvard University*	Histocompatible Pluripotent Stem Cells

Monday, January 21

Session 1: High Throughput and Microfluidic Screening Platforms

Chair: Ravi Kane, *Rensselaer Polytechnic Institute*

8:30am – 9:10am	**Invited Speaker: Sangeeta Bhatia,** *Massachusetts Institute of Technology*	Engineering the Stem Cell Microenvironment: Microtechnology Tools for High-Throughput Analysis
9:10am – 9:30am	**Daniel G. Anderson,** *Massachusetts Institute of Technology*	Combinatorial Development of Biomaterials for Tissue Engineering and Drug Delivery
9:30am – 9:50am	**Tiago G. Fernandes,** *Rensselaer Polytechnic Institute*	A High Throughput 3D Cell Microarray to Study Stem Cell Fate
9:50am – 10:10am	**Shuichi Takayama,** *University of Michigan*	Microfluidics for Stem Cell Biology and Therapy
10:10am – 10:30am	**Edwin Monuki,** *University of California, Irvine*	Dielectric Differences among Neural Stem Cells and Their Progeny
10:30am – 10:50am	**Break**	St. Tropez Foyer

Session 2: Stem Cells in Drug and Gene Discovery

Chair: Sangeeta Bhatia, *Massachusetts Institute of Technology*

10:50am – 11:30am	**Invited Speaker: Sheng Ding,** *The Scripps Research Institute*	A Chemical Approach to Stem Cell Biology
11:30am – 11:50am	**Jon Chesnut,** *Invitrogen*	Chromosomal Targeting in Embryonic Stem Cells Using the phiC31 Familay of Integrases: Stem Cells as Enabling Tools for Screening
11:50am – 12:10pm	**Joel Voldman,** *Massachusetts Institute of Technology*	Microscale Manipulation of Mouse Embryonic Stem Cells for Studying Cell Fusion-Mediated Reprogramming and Diffusible Signaling
12:10pm – 12:30pm	**Danny Van Noort,** *Institute of Bioengineering and Nanotechnology*	3D Cell Cultures in Microfluidics for Drug Discovery
12:30pm – 2:00pm	**Lunch**	La Terrasse
2:00pm – 2:50pm	**Keynote Speaker: Doug Lauffenburger,** *Massachusetts Institute of Technology*	Quantitative Systems Analysis of Signaling Networks Governing Cell Phenotype Behavior

Session 3: Systems-Based Approaches to Understanding Fate Decisions

Chair: Peter Zandstra, *University of Toronto*

2:50pm – 3:30pm	**Invited Speaker: Christopher Chen,** *University of Pennsylvania*	Stem Cell Differentiation: Mechanical Forces RhoA and Adhesion Signaling
3:30pm – 3:50pm	**Nicolas Plachta,** *California Institute of Technology*	Engineered Embryonic Stem Cells as a Discovery Tool in Neurobiology: Identification of Molecular Mechanisms of Axonal Degeneration
3:50pm – 4:10pm	**WeiJia Wang,** *University of Toronto*	A Multiparameter Flow Cytometric Assay to Investigate Cytokine Synergies during Erythroid Development
4:10pm – 4:30pm	**Babak Esmaeli-Azad,** *DNAmicroarray, Inc.*	Modeled Stem Cell Niche Assays Using Biomaterial Microarrays
4:30pm – 6:00pm	**Poster Session with Refreshments**	St. Tropez Foyer
	Chairs: Julie Audet, *Univerity of Toronto;* **Karen McCloskey,** *University of California at Merced;* **and Sean Palecek,** *University of Wisconsin-Madison*	
	Free Night for Dinner	

Tuesday, January 22

Session 4: Intercellular Signaling and the Engineered Niche

Chair: Sharon Gerecht, *Johns Hopkins University*

8:10am – 8:50am	**Invited Speaker: Peter Zandstra,** *University of Toronto*	Control of Auto-Regulatory Signaling to Modulate Embryonic Stem Cell Self-Renewal and Differentiation
8:50am – 9:10am	**Krishnendu Roy,** *The University of Texas at Austin*	Engineering Intercellular Signals of Thymic Niche: Towards Generation of Functional T Cells from Stem Cells
9:10am – 9:30am	**Eleftherios Sachlos,** *Harvard University*	Embryoid Body Shell Formation Reduces Diffusive Transport of Inductive Biochemicals
9:30am – 9:50am	**Basha Stankovich,** *University of California, Merced*	Role of Adhesion Molecules in Hematopoietic and Endothelial Commitment of Muring Embryonic Stem Cells
9:50am – 10:10am	**Natesh Parashurama,** *Harvard Medical School*	Activin Alters the Kinetics of Endoderm Induction in Embryonic Stem Cells Cultured on Collagen Gels
10:10am – 10:30am	**Break**	St. Tropez Foyer

Session 5: Novel Approaches for Embryonic Stem Cell Growth and Differentiation

Chair: Ralph Brandenburger, *Geron Corporation*

10:30am – 11:10am	**Invited Speaker:** Mick Bhatia, *McMaster University*	Controlling Human Embryonic Stem Cells by Niche Regulation
11:10am – 11:30am	Emmanuel E. Baetge, *Novocell, Inc.*	Engineering Embryonic Stem Cells to Create a Cell Therapy for Diabetes
11:30am – 11:50am	Clark K. Colton, *Massachusetts Institute of Technology*	Differentiation of Murine Embryonic Stem Cells to Cardiomyocytes Is Increased Under Reduced Oxygen
11:50am – 12:10pm	Jennifer Blundo, *Stanford University*	Molecular Imaging of Cardiac Constructs Derived from Human Embryonic Stem Cells
12:10pm – 12:30pm	Shelly Sakiyama-Elbert, *Washington University*	The Effect of Controlled Growth Factor Delivery on Embryonic Stem Cell Differentiation inside of Fibrin Scaffolds
12:30pm – 2:00pm	**Lunch**	La Terrasse

Session 6: Novel Approaches for Adult Stem Cell Growth and Differentiation

Chair: Irina Conboy, *University of California, Berkeley*

2:00pm – 2:40pm	**Invited Speaker:** Fred Gage, *Salk Institute*	Neurogenesis in the Adult Mammilian Brain
2:40pm – 3:00pm	Pin Wang, *University of Southern California*	Engineering Stromal Cells for Supportive Differentiation of TCR-Transduced Hematopoietic Stem Cells into CD4 T Cells *in Vitro*
3:00pm – 3:20pm	Irina Conboy, *University of California, Berkeley*	Understanding Bio-Chemical Changes in the Aged Muscle Niche
3:20pm – 3:40pm	Kartik Subramanian, *University of Minnesota*	Directed Differentiation of Multipotent Adult Progenitor Cells into the Cells of the Hepatic Lineage
3:40pm – 4:00pm	C. Lobato da Silva, *National Institute of Health*	Maximization of the Ex-Vivo Expansion of Human Hematopoietic Stem/Progenitor Cells by Direct Contact Culture with Mesenchymal Stem Cells
4:00pm – 5:30pm	**Poster Session with Refreshments**	St. Tropez Foyer
	Chairs: Julie Audet, *Univerity of Toronto;* Karen McCloskey, *University of California at Merced;* and Sean Palecek, *University of Wisconsin-Madison*	
5:30pm – 6:30pm	**Keynote Address:** Irving Weissman, *Stanford University*	Normal and Neoplastic Stem Cells
6:30pm – 9:30pm	**Dinner**	St. Tropez C, D

Wednesday, January 23

Session 7: Biomaterials for Stem Cell Growth and Differentiation

Chair: Shelly Sakiyama-Elbert, *University of Washington, St. Louis*

8:30am – 9:10am	**Invited Speaker:** Dennis Discher, *University of Pennsylvania*	Matrix Elasticity Directs Stem Cell Lineage Specification
9:10am – 9:30am	Kevin Healy, *University of California, Berkeley*	High Throughput Identificaiton of Novel Peptide Ligands for Engineering Surfaces for Stem Cells
9:30am – 9:50am	Sean P. Palecek, *University of Wisconsin - Madison*	3D Microwell Array Culture of Human Embryonic Stem Cells Reveals Effects of Colony Morphology on Cell Growth and Differentiation
9:50am – 10:10am	Todd McDevitt, *Georgia Institute of Technology / Emory University*	Microparticle Delivery of Morphogenic Factors within Embryoid Bodies for Directed Embryonic Stem Cell Differentiation
10:10am – 10:30am	David Brafman, *University of California, San Diego*	Arrayed Cellular Microenvironments for Exploring Cell Fate
10:30am – 10:50am	**Break**	St. Tropez Foyer

Session 8: Bioreactors and Bioprocesses for Cell Expansion and Differentiation

Chair: Joachim Cabral, *Institute for Biotechnology and Bioengineering, Portugal*

10:50am – 11:30am	**Invited Speaker:** Ramkumar Mandalam, *Cellerant Therapeutics*	Novel Universal Cell-Based Drug from Adult Hematopietic Stem Cell
11:30am – 11:50am	Matt Croughan, *Keck Graduate Institute*	Manufacturing Stem Cells: Lessons Learned from 15 Years of CHO Cell Culture for Recombinant Protein Production
11:50am – 12:10pm	Behnam A. Baghbaderani, *University of Calgery*	Large-Scale Production of Human Neural Precursor Cells in Computer-Controlled Suspension Bioreactors and Applications to the Treatment of Neuropathic Pain
12:10pm – 12:30pm	Rosario Scott, *Merck*	A Platform for Predictive Bioprocessing of Autologous Cells for Therapy Based on Automated Microwell Cultures
12:30pm – 2:00pm	**Lunch**	La Terrasse

Session 9: Biomterial Scaffolds for Stem Cell Therapeutic Applications

Chair: Kevin Healy, *University of California, Berkeley*

2:00pm – 2:40pm	**Invited Speaker:** Jennifer Ellisseff, *Johns Hopkins University*	Adult and Embryonic Stem Cells in Musculoskeletal Tissue Repair
2:40pm – 3:00pm	Song Li, *University of California, Berkeley*	Nanofibrous Scaffolds and Mesenchymal Stem Cells for Vascular Regenration
3:00pm – 3:20pm	Michael Sacks, *University of Pittsburgh*	Tissue-to-Cellular Deformation Coupling in Cell-Microintegrated Elastomeric Scaffolds
3:20pm – 3:40pm	Paul. Critser, *Indiana University School of Medicine*	Engineered Three-Dimensional Collagen-Based Extracellular Matrices Provide Microenvironmental Guidance of Endothelieal Colony Forming Cell Proliferation Potential and Vascular Network Formation *in Vitro*
3:40pm – 4:00pm	Sherry L. Voytik-Harbin, *Purdue University*	Fibril Microstructure-Mechanical Design Features of Engineered 3D Collagen-Based Extracellular Matrices Modulate Mesenchymal Stem Cell Proliferation and Lineage Specific Differentiation
4:00pm – 4:15pm	**Closing Remarks by** David Schaffer, *Conference Chair*	St. Tropez C,D

1. 3-D Hydrogel Microenvironments for Liver Progenitor Cells
   Gregory Underhill, Alice Chen and Sangeeta Bhatia
   Massachusetts Institute of Technology

2. A Novel Treatment of TEL/AML1 Induced Acute Lymphoblastic Leukemia Using siRNA Expressing Autologous Stem Cell Transplantation in Patients
   Vedant Arun[1] and Harpreet Kaur[2]
   [1] University of Toronto
   [2] Institute of Chemical Technology

3. AC Electrokinetic Microstirring for Enhancing Stem Cell Growth Cultures
   Hope Feldman and Carl D. Meinhart
   University of California Santa Barbara

4. An AFM Approach for Studying Human Embryonic Stem Cell Differentiation
   Rong Wang, Dengli Qiu, Jialing Xiang, Zhaoxia Li, Indumathi Sridharan and Aparna Krishnamoorthy
   Illinois Institute of Technology

5. Baculovirus as a New Gene Delivery Vector for Stem Cell Engineering and Bone Tissue Engineering
   Ching-Kung Chuang[1], Tzu-Chen Yen[2], Shu-Ju Tu[3], Huang-Chi Chen[1], Kun-Ju Lin[2], Wei-Chao Huang[2] and Yu-Chen Hu[1]
   [1] National Tsing Hua University
   [2] Chang Gung Memorial Hospital
   [3] Chang Gung University

6. BMP2, FGF2, and Dlx3 in Tissue-Specific Dental Stem Cell Differentiation
   Smit Dangaria
   University of Illinois, Chicago

7. Characterization and *in Vivo* Endocrine Differentiation of Mesenchymal Islet-Derived Precursor Cells
   Laertis Ikonomou[1], Behrous Davani[1], Bruce M. Raaka[1], Elizabeth Geras-Raaka[1], Russell A. Morton[2], Bernice Marcus-Samuels[1] and Marvin C. Gershengorn[1]
   [1] NIDDK, NIH
   [2] NIAAA, NIH

8. Controlling Early Human Embryonic Cell Fate through Manipulation of Exogenous and Endogenous Signaling
   Lawrence H. Lee, Raheem Peerani, Eugenia Kumacheva and Peter W. Zandstra
   University of Toronto

9. Development of a Defined Medium for the Expansion of Human Mesenchymal Stem Cells for Use in Clinical Applications
   Sunghoon Jung, Arindom Sen and Leo A. Behie
   University of Calgary

10. Development of Artificial Hematopoietic Stem Cell Niche *in-Vitro* Using Biomaterial Microarrays
    Babak Esmaeli-Azad[1], Catherine Howell[1], Ewa Carrier[2] and Walter Taddey[3]
    [1] DNAmicroarray, Inc.
    [2] University of California, San Diego
    [3] Hospital De Clinica

11. Directed Differentiation Using Applied Physical Forces
    Taby Ahsan and Robert M. Nerem
    Georgia Institute of Technology

12. Effects of Membrane Cholesterol on Elongation of Human Mesenchymal Stem Cells Grown in Topographically Patterned Surfaces
    So Hyun Kim[1], G. Lee[2], Seong-Won Nam[1] and Sungsu Park[1]
    [1] Ewha Women's University
    [2] Ajou University School of Medicine

13. Evaluation of Differentiation Methods Using Embryonic Stem Cells with Endothelial Specific Markers
    Saejeong Kim and Horst A. Von Recum
    Case Western Reserve

14. Expansion and Neural Commitment of Mouse Embryonic Stem Cells on a Microarray Platform
    Tiago G. Fernandes[1], Seok-Joon Kwon[1], Moo-Yeal Lee[2], Maria Margarida Diogo[3], Cláudia Lobato da Silva[2], Douglas S. Clark[4], Joaquim M.S. Cabral[3] and Jonathan S. Dordick[1]
    [1] Rensselaer Polytechnic Institute
    [2] Solidus Biosciences
    [3] Instituto Superior Técnico
    [4] University of California, Berkeley

15. Fabricating 3-D Hydrogel Scaffolds Using Stereolithography for Stem-Cell Differentiation
    Vincent Chan[1], Piyush Bajaj[1], Hjalti Sigmarsson[1], William J. Chappell[1] and Rashid Bashir[2]
    [1] Purdue University
    [2] University of Illinois, Urbana-Champaign

16. Functional Characterization of Contractile Properties of Human Embryonic Stem Cell-Derived Cardiomyocytes
    Jeffrey G. Jacot[1], Karen Wei[1], Jeffrey H. Omens[1], Andrew D. McCulloch[1] and Mark Mercola[2]
    [1] University of California, San Diego
    [2] The Burnham Institute for Medical Research

17. Gaussia Luciferase – A Novel Bioluminescent Reporter for Tracking Stem Cells Survival, Proliferation and Differentiation *in Vivo*
    Rampyari Raja Walia[1] and Bakhos A. Tannous[2]
    [1] Pluristem Innovaitons
    [2] Harvard Medical School/Massachussetts General Hospital

18. High Efficiency Generation of Epithelial Progenitors from Human Embryonic Stem Cells under Defined Conditions
    Christian M. Metallo, Lin Ji, Juan J. de Pablo and Sean P. Palecek
    University of Wisconsin-Madison

19. High-Throughput Screening of Gene Function in Stem Cells Using Clonal Microarrays
    Randoph S. Ashton[1], Joseph Peltier[2], Analeah O'Neil[2], Christopher Fasano[3], Sally Temple[3], David V. Schaffer[2] and Ravi Kane[1]
    [1] Rensselaer Polytechnic Institute
    [2] University of California, Berkeley
    [3] Albany Medical College

20. Human Mesenchymal Stem Cell Construct Development in a 3-D Perfusion Bioreactor System: Effects of Interstitial Flow and Morphogen Distribution
    Teng Ma[1], Feng Zhao[2], Katelyn Sellgren[2] and Ravi Chella[2]
    [1] Department of Chemical & Biomedical Engineering, FAMU-FSU College of Engineering
    [2] Chemical & Biomedical Engineering, FAMU-FSU College of Engineering

21. Human Mesenchymal Stem Cell Differentiation in Response to Matrix Stiffness and Transforming Growth Factor-β1 May Be Regulated by HDAC Activity
    Jennifer Park, An-Chi Tsou, Julia Chu and Song Li
    University of California, Berkeley

22. Human Mesenchymal Stem Cells Gene Expression of Osteogenic Markers in a 3D Environment
    Sílvia J. Bidarra[1], Cristina C. Barrias[1], Mário A. Barbosa[1],

Raquel Soares[2] and Pedro L. Granja[1]
[1] INEB - Instituto de Engenharia Biomédica
[2] University of Porto

23. Hydrodynamic Mixing Conditions Imposed by Rotary Orbital Culture Modulate Embryonic Stem Cell Differentiation
Todd McDevitt[1], Carolyn Y. Sargent[2], Geoffrey Y. Berguig[2] and Richard L. Carpenedo[2]
[1] Wallce H. Coulter Department of Biomedical Engineering Georgia Institute of Technology
[2] Georgia Institute of Technology / Emory University

24. Hypoxia Prolongs the *in Vitro* Lifespan of Human Mesenchymal Stem Cells and Modulates Cell-Cell Interactions
Teng Ma[1], Warren Grayson[2], Bruce Bunnell[1], and Feng Zhao[3]
[1] Florida State University
[2] Columbia University
[3] Tulane University

25. Imaging Differentiation-Induced Embryonic Stem Cells
Ann-Marie Broome[1], Edgardo Rivera[2], Saejeong Kim[1], James P. Basilion[1] and Horst A. Von Recum[1]
[1] Case Western Reserve University
[2] University of Puerto Rico

26. Inducible Enzyme Replacement in the MPSII Brain via Microcapsule-Based Delivery of Genetically Engineered Neural Stem Cells
Sasha H. Bakhru[1], Daniel Delubac[1], Christopher Highley[1], Shantanu Ganguly[1], Eda Altiok[1], Usha Kuppuswamy[1], Raymond Sekula[2], Hai-Quan Mao[3], Jonathan Jarvik[1] and Stefan Zappe[1]
[1] Carnegie Mellon University
[2] Allegheny General Hospital
[3] Johns Hopkins University

27. Magnetic Resonance Microscopy for Monitoring Stem Cell Regeneration in Vitro
Xu Feng, Richard Magin and Liu Hong
University of Illinois at Chicago

28. Molecular Mechanisms of Adult Neural Progenitor Proliferation and Self-Renewal
Joseph Peltier, Analeah O'Neill and David V. Schaffer
University of California, Berkeley

29. Mouse Embryonic Stem Cell Expansion in a Microcarrier-Based Stirred Culture System
Ana Margarida Fernandes[1], Tiago G. Fernandes[1], Maria Margarida Diogo[1], Cláudia Lobato da Silva[1], Domingos Henrique[2] and Joaquim M.S. Cabral[1]
[1] Instituto Superior Técnico
[2] IMM-Instituto de Medicina Molecular

30. Nanoparticles for Multimodal Tracking of Implanted Neural Stem Cells *in Vivo*
Sasha H. Bakhru, Eda Altiok and Stefan Zappe
Carnegie Mellon University

31. Niche-Mediated Control of Human Embryonic Stem Cell Self-Renewal and Differentiation
Raheem Peerani[1], Balaji Rao[2], Celine Bauwens[1], Ting Yin[1], Eugenia Kumacheva[1] and Peter W. Zandstra[1]
[1] University of Toronto
[2] North Carolina State University

32. Propagation of Embryonic Stem Cells as Pluripotent Aggregates in Stirred-Suspension Vessels without Serum
Daniel E. Kehoe, Lye T. Lock, and Emmanuel (Manolis) S. Tzanakakis
State University of New York at Buffalo

33. Protein Coated Scaffold for the Differentiation of Type II Pneumocytes from Murine Embryonic Stem Cells
Yuan-Min Lin, Anne Bishop, and Alexander Bismarck
Imperial College London

34. Reversible Biomolecule Self-Assembly and Presentation on Biomaterials Surfaces
Travis J. Sill and Horst A. Von Recum
Case Western Reserve University

35. Role of p27 in Cyclic Adenosine Monophosphate Caused Differentiation in Rat Mesenchymal Stem Cells
Linxia Zhang and Christina Chan
Michigan State University

36. Selective Tenocyte Differentiation of Mesenchymal Stem Cells Using Bone Morphogenetic Protein-12
Yonghui Li[1], Hui B. Sun[1], David T. Fung[1], Melissa Ramcharan[2], Robert J. Majeska[1], Mitchell B. Schaffler[1], Evan L. Flatow[1] and Yuling Li[3]
[1] Mount Sinai School of Medicine
[2] CCNY/CUNY Graduate Center
[3] Jilin University

37. Serum-Free Derivation and Expansion of Endothelial from Embryonic Stem Cells
Alicia Blancas and Kara McCloskey
University of California, Merced

38. Synergistic Acceleration of Stem Cell Mediated Heart Valve Tissue Formation by Cyclic Flexure and Laminar Flow
George Engelmayr[1], Michael Sacks[2], and John Mayer Jr[3]
[1] Massachusetts Institute of Technology
[2] University of Pittsburgh
[3] Children's Hospital Boston

39. The Effect of Cell Density and Hypoxia on Potency of Rodent Multipotent Adult Progenitor Cells (MAPCs)
Yonsil Park[1], Fernando Ulloa-Montoya[2], Catherine Verfaillie[2], and Wei-Shou Hu[1]
[1] University of Minnesota
[2] Katholieke Universiteit

40. Tracking of Oligodendrocyte Remyelinated Axons in Spinal Cords
Joerg Meyer, Kristine Velasco and M. Gopi
University of California, Irvine

41. Transcriptional Profiling to Understand Genomic Instability during Human Embryonic Stem Cell Propagation
Raj R. Rao, Marion Joe Riggs, and Venkat Sundar Gadepalli
[1] Virginia Commonwealth University

42. Tuning Hydrogel Modulus and Ligand Density Independently to Control Adult Neural Stem Cell Self-Renewal and Differentiation
Kevin E. Healy, David V. Schaffer, and Krishanu Saha
University of California, Berkeley

43. Ultra-High-Throughput Production of Highly Organized and Reproducible Human Embryoid Bodies
Mark D. Ungrin, Chirag Joshi, Celine Bauwens and Peter W. Zandstra
University of Toronto

INSTRUCTIONS

SPEAKERS

Speakers should plan to meet the session chair at least 15 minutes prior to the session. Please sit in the front of the room during your session.

Your presentation must be uploaded to the conference computer at least 30 minutes before the session. The presentation needs to be in either Powerpoint or pdf format.

Contributed speakers will have 20 minutes for their talk, including questions. Invited speakers will have 40 minutes, including questions. Please help us remain on time.

POSTER PRESENTERS

Merck Research Laboratories has generously provided poster awards, which will be presented on Wednesday, January 23rd. Please set up your poster in the St. Tropez Salon A and B on Monday morning, January 21st. Odd posters will be presented on Monday, January 21st, and even posters will be presented on Tuesday, January 22nd. Posters must be taken down after the reception on Tuesday, January 22.

Exhibitors

Exhibitors should set up your booth or table top on Sunday afternoon, January 20th, from 3-5pm. Exhibitors should take down their booth after the break on Wednesday, January 23rd. Exhibit hours will be during breakfasts, session breaks, and all conference receptions.

IMPORTANT ADDRESSES

SBE staff will be available at the Registration Booth in the St. Tropez Foyer at the following times:

Sunday, January 20	3pm-6pm
Monday, January 21	7am-4pm
Tuesday, January 22	7am-3pm
Wednesday, January 23	7am-10am

Following the conference, you may reach SBE's Executive Director, June Wispelwey, by email at junew@aiche.org or by phone at 610-291-4036.

CONFERENCE CHAIRS

FRED H. GAGE, PH.D.

The Salk Institute

Fred H. Gage, Ph.D., a Professor in the Laboratory of Genetics, joined The Salk Institute in 1995. He received his Ph.D. in 1976 from The Johns Hopkins University. Dr. Gage's work concentrates on the adult central nervous system and unexpected plasticity and adaptability to environmental stimulation that remains throughout the life of all mammals. In addition, his studies focus on the cellular, molecular, as well as environmental influences that regulate neurogenesis in the adult brain and spinal cord.

Prior to joining Salk, Dr. Gage was a Professor of Neuroscience at the University of California, San Diego. He is a Fellow of the American Association for the Advancement of Science, a Member of the National Academy of Sciences and the Institute of Medicine, and a Member of the American Academy of Arts and Sciences. Dr. Gage also served as president of the Society for Neuroscience in 2002.

In addition, Dr. Gage has been the recipient of numerous prestigious awards, among them the 1993 Charles A. Dana Award for Pioneering Achievements in Health and Education, the Christopher Reeve Research Medal in 1997, the 1999 Max Planck Research Prize, and the MetLife Award in 2002.

DAVID SCHAFFER, PH. D.

University of California, Berkeley

Professor Schaffer is a Professor of Chemical Engineering, Bioengineering, and the Helen Wills Neuroscience Institute at the University of California, Berkeley. He graduated from Stanford University with a B.S. degree in Chemical Engineering in 1993. Afterward, he attended Massachusetts Institute of Technology and earned his Ph.D. also in Chemical Engineering in 1998 with Professor Doug Lauffenburger. While at M.I.T., Dave minored in Molecular and Cell Biology. Finally, he did a postdoctoral fellowship in the laboratory of Fred Gage at the Salk Institute for Biological Studies in La Jolla, CA, before moving to UC Berkeley in 1999. At Berkeley, Dr. Schaffer applies engineering principles to enhance stem cell and gene therapy approaches for neuroregeneration. This work includes mechanistic investigation of stem cell control, as well as molecular evolution and engineering of viral gene delivery vehicles. David Schaffer has received an NSF CAREER Award, Office of Naval Research Young Investigator Award, Whitaker Foundation Young Investigator Award, and was named a Technology Review Top 100 Innovator. He was also awarded the American Chemical Society BIOT Division Young Investigator Award in 2006 and the Biomedical Engineering Society Rita Shaffer Young Investigator Award in 2000.

KEYNOTE SPEAKERS

GEORGE Q. DALEY, M.D., PH.D.

Boston Children's Hospital
Harvard Medical School

Dr. Daley is the Associate Professor in the Division of Hematology/Oncology at Children's Hospital Boston and the Brigham and Women's Hospital and the Department of Biological Chemistry and Molecular Pharmacology at Harvard Medical School. He is Associate Director of the Stem Cell Program at Boston Children's Hospital and a member of the Executive Committee of the Harvard Stem Cell Institute. Dr. Daley is currently the President of the International Society for Stem Cell Research, and was recently named an investigator of the Howard Hughes Medical Institute. Dr. Daley's research is aimed at translating insights in stem cell biology into improved therapies for genetic diseases. His laboratory reported the first successful application of therapeutic cloning of embryonic stem cells to treat genetic disease in a mouse model of immune deficiency (together with Rudolf Jaenisch), and the first creation of sperm from embryonic stem cells, work that was cited by Science magazine as a “Top Ten” breakthrough for 2003. As a graduate student, Dr. Daley proved that the BCR/ABL oncogene causes human chronic myeloid leukemia (CML). This work validated BCR/ABL as a target for drug blockade and was a crucial step in the development of imatinib (GleevecTM; Novartis), a revolutionary magic-bullet chemotherapy that induces remissions in virtually every CML patient. Dr. Daley's recent studies have clarified mechanisms of Gleevec resistance and have helped identify novel combination chemotherapeutic regimens.

Dr. Daley received his bachelor's degree magna cum laude from Harvard University (1982) and a Ph.D. in biology from MIT (1989) working with Nobelist Dr. David Baltimore. Dr. Daley received his M.D. from Harvard Medical School, where he was only the twelfth individual in the school's history to be awarded the degree summa cum

laude (1991). He served as Chief Resident in Internal Medicine at the Massachusetts General Hospital and is currently a staff physician in Hematology/Oncology at the Children's Hospital, the Dana Farber Cancer Institute, and the Brigham and Women's Hospital in Boston. His has received awards from Harvard Medical School, the National Institutes of Health, the New England Cancer Society, the Burroughs Wellcome Fund, the Edward Mallinckrodt, Jr. Foundation, and the Leukemia and Lymphoma Society of America recognizing his contributions to medical research. He has been elected a fellow of the American Association for the Advancement of Science and a member of the American Society for Clinical Investigation. Dr. Daley was an inaugural winner of the NIH Director's Pioneer Award, which provides a five year unrestricted grant to pursue highly innovative research, and was recently awarded the Judson Daland Prize from the American Philosophical Society for achievement in patient-oriented research.

DOUGLAS LAUFFENBURGER, M.D., PH.D.

Massachusetts Institute of Technology

Douglas A. Lauffenburger is the Uncas & Helen Whitaker Professor of Bioengineering and Head of the Department of Biological Engineering at the Massachusetts Institute of Technology. Professor Lauffenburger also holds appointments in the Department of Biology and the Department of Chemical Engineering, is a member of the Biotechnology Process Engineering Center, Center for Biomedical Engineering, Center for Cancer Research, and Center for Environmental Health Sciences, and is Director of the Computational & Systems Biology Initiative.

Dr. Lauffenburger's B.S. and Ph.D. degrees are in chemical engineering from the University of Illinois and the University of Minnesota, in 1975 and 1979 respectively. His major research interests are in cell engineering: the fusion of engineering with molecular cell biology. A central focus of his research program is in receptor-mediated cell communication and intracellular signal transduction, with emphasis on development of predictive computational models derived from quantitative experimental studies, for cell cue/signal/response relationships important in pathophysiology with application to drug discovery and development. Lauffenburger has coauthored a monograph entitled Receptors: Models for Binding, Trafficking & Signaling, published by Oxford University Press in 1993 and reprinted in 1996. More than 80 doctoral students and postdoctoral associates have completed their training under his supervision or co-supervision.

Professor Lauffenburger has served as a consultant or scientific advisory board member for Astra-Zeneca, Beyond Genomics, CellPro, Eli Lilly, Entelos, Genstruct, Insert Therapeutics, Johnson & Johnson, Merrimack Pharmaceuticals, Pfizer, Precision Therapeutics, SyStemix, the Burroughs-Wellcome Fund, and the Whitaker Foundation. His awards include the Pierre Galletti Award from AIMBE, the A.P. Colburn Award, Bioengineering Division Award, and W.H. Walker Award from AIChE, the Distinguished Lecture Award from BMES, the C.W. McGraw Award from ASEE, the Amgen Award in Biochemical Engineering from the Engineering Foundation, and a J.S. Guggenheim Fellowship, along with a number of named lectures at academic institutions. He is a member of the National Academy of Engineering and of the American Academy of Arts & Sciences, and has served as President of the Biomedical Engineering Society, Chair of the College of Fellows of AIMBE, and on the Advisory Council for the National Institute for General Medical Sciences at NIH.

IRVING WEISSMAN, M.D., PH.D.

Stanford University

Dr. Weissman, M.D., is the Director of the Stanford Institute for Stem Cell Biology and Regenerative Medicine, Director of the Stanford Comprehensive Cancer Center and Director of the Stanford Ludwig Center for Stem Cell Research. Dr. Weissman was a member of the founding Scientific Advisory Boards of Amgen (1981-1989), DNAX (1981-1992), and T-Cell Sciences (1988-1992). He co-founded SyStemix in 1988, StemCells in 1996, and Celtrans (now Cellerant), the successor to SyStemix, in 2001.

He is a Director and Chair of their Scientific Advisory Boards.
His research encompasses the biology and evolution of stem cells and progenitor cells, mainly blood-forming and brain-forming. He is also engaged in isolating and characterizing the rare cancer and leukemia stem cells as the only dangerous cells in these malig-

nancies, especially with human cancers. Finally, he has a long-term research interest in the phylogeny and developmental biology of the cells that make up the blood-forming and immune systems. His laboratory was first to identify and isolate the blood-forming stem cell from mice, and has purified each progenitor in the stages of development between the stem cells and mature progeny (granulocytes, macrophages, etc.). At SyStemix he co-discovered the human hematopoietic stem cell and at StemCells, he co-discovered a human central nervous system stem cell. In addition, the Weissman laboratory has pioneered the study of the genes and proteins involved in cell adhesion events required for lymphocyte homing to lymphoid organs *in vivo*, either as a normal function or as events involved in malignant leukemic metastases.

Professor Weissman is a member of the National Academy of Sciences (1989-present), the Institute of Medicine at the National Academy (2002-present), and the American Association of Arts and Sciences (1990-present) and the American Academy of Microbiology (1997-present). He served as President of the American Association of Immunologists in 1994. He has received the Outstanding Investigator Award from the National Institutes of Health (1986), the Kaiser Award for Excellence in Preclinical Teaching (1987), the Pasarow Award in Cancer Research (1989), the Harvey Lecture Award (1989), the De Villiers International Achievement Award of the Leukemia Society of America (1999), and the E. Donnall Thomas Prize from the American Society of Hematology (1999). He received an Honorary Doctor of Science Degree from Montana State University (1992), was Selected Top 100 Alumni of Montana State University (1993), the Montana Conservationist of the Year Award (1994). Professor Weissman also received the Ellen Brown Scripps Society Medal (2001), the Irvington Institute Immunologist of the Year Award (2001), the Van Bekkum Stem Cell Award (2002), the California Scientist of the Year Award (2002), the Association of American Cancer Institutes 2002 Distinguished Scientist Award, the Basic Cell Research Award by the American Society of Cytopathology (2002), The Society of Neurological Surgeons Bass Award (2003), the J. Allyn Taylor International Prize in Medicine (2003), the American Diabetes Association Elliott Proctor Joslin Medal (2003), and the Rabbi Shai Shacknai Memorial Prize in Immunology and Cancer Research from the Lautenberg Center for General and Tumor Immunology (2004). In 2004, Dr. Weissman was awarded the New York Academy of Medicine Award for Distinguished Contributions to Biomedical Research, the Jessie Stevenson Kovalenko Medal from the National Academy of Sciences Council, and was the Alan Cranston Awardee from the Alliance for Aging Research. He received The Linus Pauling Medal for Outstanding Contributions in Science from Stanford University and the “Dare to Dream” award from the Jeffrey Modell Foundation in 2005. In 2006 Professor Weissman was awarded an Honorary Doctorate from Columbia University, the John Scott Award from the City of Philadelphia, the American Italian Cancer Foundation's Prize for Scientific Excellence in Medicine, and the Commonwealth Club of California's 18th Annual Distinguished Citizen Award. In 2007, he has been awarded an Honorary Doctorate from the Mount Sinai School of Medicine, New York City, New York and the I. &. H. Wachter Award from the I. & H. Wachter Foundation in Innsbruck, Austria.

INVITED SPEAKERS

SANGEETA N. BHATIA, M.D., PH.D.

Massachussets Institute of Technology

Dr. Bhatia is an Associate Professor at the Massachusetts Institute of Technology. Her work focuses on using micro- and nanotechnology tools to repair damaged tissues. Dr. Bhatia trained at Brown, MIT, and Harvard. After postdoctoral training at the Massachusetts General Hospital, she was a member of the Bioengineering Department at University of California at San Diego for 6 years. In 2005, she returned to Boston to join the MIT faculty. She has been awarded the David and Lucile Packard Fellowship given to 'the nation's most promising young professors in science and engineering,' the MIT TR100 Young Innovators Award, and been named one of San Diego's '50 People to Watch in 2004'. Her research portfolio includes funding from NIH, NSF, DARPA, NASA, the Whitaker Foundation, the Packard Foundation, and private industry. She co-authored the first undergraduate textbook on tissue engineering and is a frequent advisor to governmental organizations on cell-based sensing, nanobiotechnology, and tissue engineering. She holds 12 issued or pending patents and has worked in industry at Pfizer, Genetics Institute, ICI Pharmaceuticals, and Organogenesis.

CHRISTOPHER S. CHEN, M.D., PH.D.
University of Pennsylvania

Dr. Chen is the Skirkanich Associate Professor of Innovation at the University of Pennsylvania's Department of Bioengineering, a faculty member of the Cell Biology and Physiology Program and Cell Growth and Cancer Program, and Director of the Tissue Microfabrication Laboratory. Dr. Chen has been an instrumental figure in the development of engineered cellular microenvironments in order to engineer cell function. The goal of Dr. Chen's research is to identify the underlying mechanisms by which cells interact with materials and each other to build tissues, and to apply this knowledge in the biology of stem cells, tissue vascularization, and cancer. Dr. Chen has received numerous honors, including the Presidential Early Career Award for Scientists and Engineers, the Angiogenesis Foundation Fellowship, the Office of Naval Research Young Investigator Award, the Mary Hulman George Award for Biomedical Research, and the Herbert W. Dickerman Award for Outstanding Contribution to Science. He serves as a member of the Faculty of 1000 Biology, the Board of Trustees for the Society for BioMEMS and Biomedical Nanotechnology, Editor for BioInterphases and Molecular and Cellular Biomechanics, and member of the Defense Sciences Study Group. He received his A.B. in Biochemistry from Harvard, M.S. in Mechanical Engineering from M.I.T., and Ph.D. in Medical Engineering and Medical Physics from the Harvard-M.I.T. Health Sciences and Technology Program. He earned his M.D. from the Harvard Medical School. He was Assistant Professor in Biomedical Engineering and in Oncology at Johns Hopkins University prior to his current appointment.

DR. SHENG DING, PH.D.
Scripps Research Institute

Dr. Sheng Ding is the Associate Professor of the Chemistry and Cell Biology Departments at the Scripps Research Institute. He obtained his B.S. in chemistry with honors from Caltech in 1999, and a Ph.D. in chemistry from Scripps in 2003. Dr. Ding is developing and integrating chemical and functional genomic tools to study stem cell biology and regeneration, with an emphasis on high throughput cellular screening approach to identify small molecules and genes which can control cell fate.

DENNIS E. DISCHER, PH.D.
University of Pennsylvania

Dr. Discher is the Professor of the departments of Chemical & Biomolecular Engineering and Bioengineering at the University of Pennsylvania. He is coauthor of ca.150 papers and book chapters ranging from topics in single molecule and stem cell biophysics to polymer-based nano-delivery of drugs with development of polymersomes and filomicelles. The work emphasizes physical chemistry and statistical mechanics as well as molecular and cell biology, and has appeared in a wide range of journals, including Cell, Science, PNAS, and Nature Nanotechnology. Past awards include an NSF-PECASE Award, Friedrich Wilhelm Bessel Award from the Humboldt Foundation, and the Journal of Controlled Release-Best Paper Award.

JENNIFER ELISSEEFF, PH.D.
Johns Hopkins University

Dr. Elisseeff is an associate professor of biomedical engineering at Johns Hopkins University with an adjunct appointment in Orthopedic Surgery. Her biomaterials and tissue engineering laboratory at Johns Hopkins focuses on developing new biomaterials and minimally invasive technologies for tissue repair, stem cells, and musculoskeletal tissue engineering. She has collaborations with plastic surgery, orthopedics, ophthalmology and otolaryngology clinical departments. Dr. Elisseeff has published over 50 articles and book chapters, 6 patents issued and pending, and has given over 70 invited national and international lectures. She serves on a number of NIH and foundation review panels, the science advisory board for Bausch and Lomb and Cellular Bioengineering, Inc, and recently cofounded a company that is developing new materials and therapies for cartilage repair. Dr. Elisseeff has received awards including the Carnegie Mellon Young Alumni Award, Arthritis Investigator Award from the Arthritis Foundation and was named by Technology Review magazine as a top innovator under 35 in 2002 and top 10 technologies to change the future. In 2005, the Urbanite magazine voted Elisseeff one of the up and coming in Baltimore and the Baltimore Business Journal recently named her to Baltimore's 40 under 40.

FRED H. GAGE, PH.D.

The Salk Institute
University of California, San Diego

Fred H. Gage, Ph.D., a Professor in the Laboratory of Genetics, joined The Salk Institute in 1995. He received his Ph.D. in 1976 from The Johns Hopkins University. Dr. Gage's work concentrates on the adult central nervous system and unexpected plasticity and adaptability to environmental stimulation that remains throughout the life of all mammals. In addition, his studies focus on the cellular, molecular, as well as environmental influences that regulate neurogenesis in the adult brain and spinal cord.

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In addition, Dr. Gage has been the recipient of numerous prestigious awards, among them the 1993 Charles A. Dana Award for Pioneering Achievements in Health and Education, the Christopher Reeve Research Medal in 1997, the 1999 Max Planck Research Prize, and the MetLife Award in 2002.

RAMKUMAR MANDALAM, PH.D.

Cellerant Therapeutics, Inc.

Ramkumar Mandalam, Ph.D. is currently Chief Operating Officer at Cellerant Therapeutics Inc, a biotechnology company in San Carlos, CA, developing products for the treatment of hematopoietic disorders. Prior to joining Cellerant in 2005, he was the Executive Director of Product Development at Geron Corporation, a biopharmaceutical company in Menlo Park, CA. His responsibility at Geron included strategic planning and implementation of development and manufacturing of cellular products for 'regenerative medicine', oncology and drug discovery applications. Dr. Mandalam's expertise and interest is in the areas of R & D, manufacturing and commercialization of human cellular products and biopharmaceuticals. Prior to joining Geron in 2000, he was Director of Developmental Research at Aastrom Biosciences managing research and development programs involving ex vivo expansion of human primary cells (including bone marrow and cord blood cells) for cell therapy applications. Dr. Mandalam received his B.Tech (Chem Eng.) from India, M.S. (Chem Eng.) and Ph.D (Chem Eng.) from University of Michigan, USA.

PETER W. ZANDSTRA, PH.D.

University of Toronto

Dr. Peter Zandstra graduated with a Bachelor of Engineering degree from McGill University in the Department of Chemical Engineering, obtained his Ph.D. degree from the University of British Columbia in the Department of Chemical Engineering and Biotechnology, and carried out his research training as a Post Doctoral Fellow in the field of Bioengineering at the Massachusetts Institute of Technology. Dr. Zandstra joined the faculty at the University of Toronto in 1999, where is he is a professor in the Institute of Biomaterials and Biomedical Engineering (with cross-appointments to Departments of Chemical Engineering and Applied Chemistry and Medical Genetics). Dr Zandstra is the Canada Research Chair in Stem Cell Bioengineering and is a recipient of awards such as the Guggenheim Fellowship (2007), the Premiers Research Excellence Award and the E.W.R. Steacie Memorial Fellowship. In 2007 elected as fellow of the American Association for the Advancement of Science.

Research in Zandstra's Stem Cell Bioengineering Laboratory is focused on understanding the interface between microenvironmental control and the endogenous (niche-mediated) and intracellular networks that underlie stem cell fate decisions. We are motivated by the hypothesis that appropriate engineering of the cellular microenvironment will enable robust and efficient manipulation of stem cell self-renewal and differentiation. Using bioengineering strategies such as predictive mathematical modeling, microfabrication and bioreactors, our work is applied to three project areas: quantitative, spatial and temporal control of embryonic stem cell self-renewal; bioprocesses for the generation of blood and cardiac cells from embryonic stem cells; and control of intercellular signaling networks to grow human blood stem cells. Ultimately, our goal is to enable stem cell based therapies and technologies and thus positively impact upon health and welfare.

KEYNOTE PRESENTATION

Histocompatible Pluripotent Stem Cells

George Q. Daley
Department of Biological Chemistry and Molecular Pharmacology, Children's Hospital Boston, Harvard Medical School and Harvard Stem Cell Institute, 300 Longwood Avenue Karp-7, Boston, MA 02115

Embryonic stem (ES) cells represent an inexhaustible source of precursor cells that can be differentiated into specific cell lineages. As with conventional organ transplants, ES cell-based therapies will face immunologic barriers. Genetically matched pluripotent embryonic stem cells generated via nuclear transfer (ntES cells), parthenogenesis (pES cells), or induced by defined factors (iPS cells) are a possible source of histocompatible cells and tissues. In a proof of principle experiment, we have shown that customized ntES cells can be used to repair a genetic immunodeficiency disorder in mice (Rideout et al., Cell 2002). However, generation of ES cells by nuclear transfer remains inefficient, and to date has not been achieved with human cells. ES cells with defined histocompatibility loci can be generated at much higher efficiency by direct parthenogenetic activation of the unfertilized oocyte (Kim et al., Science 2007). Subsequently, cell lines can be genotyped and selected for MHC identity to the oocyte donor. Cell lines with homozygous MHC haplotypes can also be identified, and tissues from such cells engraft in MHC heterozygous recipients. Compared to ES cell lines from fertilized embryos, pES cells display comparable *in vitro* hematopoietic activity, and blood derivatives can repopulate hematopoiesis in irradiated adult mouse recipients. These experiments establish murine models for generating histocompatible ES cell-derived tissue products, and suggest the theoretical feasibility of ES cell banking to enable off-the-shelf cell therapies. We have generated human iPS cells by direct reprogramming of human somatic cells with retroviruses carrying OCT4, SOX2, MYC, and KLF4. Although this represents a platform for generating customized, patient-specific cells for research, methods must be established for making human iPS cells that do not entail viral transgenesis. Therefore, current efforts are also aimed at applying interspecies nuclear transfer and parthenogenesis to generate genetically unmodified pluripotent human stem cells.

SESSION 1: HIGH THROUGHPUT AND MICROFLUIDIC SCREENING PLATFORMS

Invited Presentation

Engineering the Stem Cell Microenvironment: Microtechnology Tools for High-Throughput Analysis

Sangeeta Bhatia
Massachusetts Institute of Technology, Division of Medicine, Brigham and Women's Hospital, Harvard-MIT Division of Health Sciences and Technology/Electrical Engineering and Computer Science, 77 Massachusetts Ave., Building E19-502d, Cambridge, MA 02139

The ability to engineer tissue has applications in the development of both cell-based therapies and *in vitro* model systems that can be used to study *in vivo* processes. Stem cells are uniquely positioned at the foundation of potential regenerative medicine therapies because of their distinctive ability to undergo self-renewal combined with the capacity to generate numerous differentiated cell types, including progenitor and effector cell populations. We have developed a repertoire of microtechnology tools to control and study the role of cell-extracellular matrix (ECM) interactions, cell-cell interactions, soluble stimuli, and three-dimensional context on cellular function. For example, to investigate cell-ECM interactions, we have utilized a microarray cell culture platform and examined the effects of combinations of ECM and their crosstalk with growth factors. We have also further explored soluble stimuli through the application of microfluidic systems, and have employed a microfluidics approach to develop a multiplexed timelapse imaging platform. In this system, cells within many isolated environments can be evaluated through near-simultaneous timelapse imaging over the course of many days. Furthermore, to specifically aid in the clonal analysis of stem cells, we have demonstrated the utility of a microfabricated multi-well platform for cell fate analysis. Finally, in the realm of three-dimensional microenvironments we have utilized synthetic hydrogel-based approaches that enable manipulation of both the chemical environment and multicellular architecture of cells in 3D. These include systems for multiplexed parallel analysis of 3D constructs and *in vivo* analysis of cellular responses. We anticipate that a combination of robust tools to manipulate stem cells with insight on the role of their microenvironment should have broad clinical and technological implications.

Oral Presentation

Combinatorial Development of Biomaterials for Tissue Engineering and Drug Delivery

Daniel G. Anderson
Center for Cancer Research, Massachusetts Institute of Technology, 77 Massachusetts Ave., Room E25-342, Cambridge, MA 02139

High throughput, combinatorial approaches have revolutionized small molecule drug discovery. Here we describe our work on high throughput methods for developing and characterizing biomaterials for drug delivery and tissue engineering. We have developed automated methods allowing for rapid nanoliter scale synthesis of 1000's of biomaterials, as well as the testing of their chemical, material, and biological properties. These methods have also been applied towards the development of new methods to control stem cell behavior, as well as vehicles for drug delivery. In particular, these combinatorial libraries of different biomaterials have enabled new methods for microparticulate drug delivery, non-viral gene therapy, siRNA delivery, and vaccines.

Oral Presentation

High-Throughput 3D Cell Microarray to Study Stem Cell Fate

Tiago G. Fernandes[1], Seok Joon Kwon[1], Moo-Yeal Lee[2], Maria Margarida Diogo[3], Cláudia Lobato da Silva[3], Douglas S. Clark[4], Joaquim M.S. Cabral[3] and Jonathan S. Dordick[1]
[1] Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 110 Eighth Street, Troy, NY 12180,
[2] Solidus Biosciences, 1223 Peoples Avenue, Troy, NY 12180
[3] IBB - Institute for Biotechnology and Bioengineering, CEBQ, Instituto Superior Técnico, Avenida Rovisco Pais, Lisbon, 1049-001, Portugal
[4] Chemical Engineering, University of California - Berkeley, Berkeley, CA 94720

Microscale technologies are emerging as powerful tools for tissue engineering and high-throughput screening, and as cell-based probes in chemical biology. With the advent of robotic spotting technology, it is now possible to distribute nanoliter samples in a spatially addressable manner. As a result, cell-based microarrays can enable high-throughput parallel screening of a large number of small molecules. A particularly promising application of such cell-based microarrays is for the growth and differentiation of stem cells, and for investigating the influence of small molecules and cell growth conditions on cell physiology and function.

We are developing microarray platforms that enable the rapid and efficient tracking of stem cell fate and quantification of specific stem cell markers. These capabilities may contribute to a better understanding of the cellular mechanisms involved in stem cell expansion and differentiation, and may enable the development of high-throughput cell-based screening devices for rapid and effective identification of small molecules that can be used to direct cellular responses. In the present work we have developed a miniaturized 3D cell culture chip for high-throughput screening. A non-contact microarrayer was used to spot mouse embryonic stem (mES) cells on poly(styrene-co-maleic anhydride) spin-coated glass slides to yield an array that consists of mES cells encapsulated in alginate gel spots with volumes as low as 20 nL.

Preliminary results revealed that this platform is suitable for studying the expansion of mES cells, as they retain their pluripotent and undifferentiated state. The cells remained highly viable and exhibited morphology typical of an undifferentiated state, forming highly compact cell clusters inside the hydrogel spots. The fold increase in total cell number ranged between 17 ± 5 for lower initial cell loadings (50 cells per spot), and 5 ± 1 for higher initial cell densities (400 cells per spot). Kinetic parameters such as the specific growth rate and the doubling time were also calculated as a function of cell density. A maximum specific growth rate of 1.0 ± 0.3 day-1 was obtained, which was similar to that observed in culture plates and spinner flasks. Thus, scale reduction of over 106-fold did not affect cell growth.

A critical task in studying cell function is the analysis of protein expression. To that end, we have developed a method based on an immunostaining technique scaled down to function on a cellular microarray. This on-chip, in-cell Western analysis allows quantification of the levels of specific cell marker proteins on a microarray. Specifically, following five days of expansion in serum-free medium containing leukaemia inhibitory factor (LIF) and bone morphogenic protein-4 (e.g., undifferentiating conditions), the levels of the target protein Oct-4 were quantified and compared with the levels obtained for cells growing in differentiating conditions (e.g., in serum-containing medium without LIF). The protocol was able to distinguish these two cell populations based on this marker of pluripotency and significantly higher levels of Oct-4 were obtained for cells grown in undifferentiating conditions. We also examined neural commitment of mES cells on the microarray. We observed the generation of neuroectodermal precursor cells, which were characterized by expression of the early neuronal marker Sox-1, whose levels were also measured *in situ* using a green fluorescent protein reporter system.

We envision that the 3D stem cell microarray platform can be further extended to applications involving protein and drug candidate screening, enzyme inhibition, and cytotoxicity. Thus, we expect this work to impact the design and control of stem cells for tissue engineering and biological studies, as well as afford potential drug discovery assays in the biotechnology and pharmaceutical industries.

Oral Presentation

Microfluidics for Stem Cell Biology and Therapy

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Many stem cell studies and cellular therapies require culture and manipulation of small numbers of highly environment-sensitive cells. The gap between the cellular microenvironment *in vivo* and *in vitro*, however, poses challenges for obtaining physiologically relevant responses from the stem cells. One of the reasons for this gap is because the fluidic environment of mammalian cells *in vivo* is microscale and dynamic whereas typical *in vitro* cultures are macroscopic and static. Another reason is that conventional cultures are often two-dimensional (2D) whereas physiological environments are 3-D. This presentation will give an overview of efforts in our laboratory to develop programmable microfluidic systems that enable spatio-temporal control of both the chemical and fluid mechanical environment of cells. The presentation will also discuss methods to form and manipulate microscale 3D cell cultures within the microfluidic devices. The technologies and methods close the physiology gap to provide biological information otherwise unobtainable and to enhance cellular performance in therapeutic applications. Specific biomedical topics that will be discussed include microfluidic embryo and embryoid body manipulation, studies of the effect of physiological and pathological fluid mechanical stresses on cellular responses. Time permitting, use of tunable nanofluidic systems for single molecule genetic analysis will also be discussed.

Oral Presentation

Dielectric Differences among Neural Stem Cells and Their Progeny

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Stem cell biology and therapies are currently hampered by a lack of adequate cell sorting tools. Cell-type specific markers, such as cell surface proteins used for flow cytometry or FACS, are limited and often recognize multiple members of stem cell lineages. To develop a complementary 'label-free' approach that would not require prior knowledge of cell surface epitopes, we used microfluidics and dielectrophoresis (DEP), which induces a frequency-dependent dipole in cells. We found that populations of mouse neural stem/precursor cells (NSPCs), differentiated neurons, and differentiated astrocytes have markedly different dielectric properties, as revealed by DEP. By isolating NSPCs known to have different neuron or astrocyte fate biases based on developmental stage, we also demonstrate shifts in DEP curves that reflect NSPC fate bias and precede detectable differences in marker expression. Additionally, experimental data and mathematical modeling suggest that DEP curve slope serves as an accurate and sensitive indicator of cell heterogeneity in mixed cultures. These findings provide evidence for a more global cell property that reflects stem cell fate bias, and establish DEP as a tool with unique capabilities for interrogating, characterizing, and sorting stem cells and their differentiated progeny.

SESSION 2: STEM CELLS IN DRUG AND GENE DISCOVERY

Invited Presentation

A Chemical Approach to Stem Cell Biology

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Recent advances in stem cell biology may make possible new approaches for the treatment of a number of diseases. A better understanding of the molecular mechanisms that control stem cell fates as well as an improved ability to manipulate them are required. Toward these goals, we have developed and implemented chemical and functional genomic tools,

including high throughput cell-based phenotypic screens of arrayed chemical, cDNA and RNAi libraries, genomic and proteomic profiling of homogenous undifferentiated/self-renewing or selectively differentiated cell populations under chemically defined conditions, and in-depth biochemical and functional assays *in vitro* and *in vivo*, to identify and further characterize small molecules and genes that can control stem cell fate in various systems. This talk will provide specific examples of stem cell discovery efforts in my lab that have advanced our ability and understanding toward controlling embryonic stem cell fate.

Oral Presentation

Chromosomal Targeting in Embryonic Stem Cells Using the phiC31 Family of Integrases: Stem Cells as Enabling Tools for Screening

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One of the biggest obstacles to using human embryonic stem cells in the generation of assay platforms is the difficulty in achieving stable, homogeneous expression of lineage reporters and cell perturbation constructs in these cells in culture. Our group is working towards developing genetically engineered hESC lines as a unique platform that will enable isolation of homogenous populations of cells containing multiple genetic elements inserted in a single, defined chromosomal locus. This system uses lambda phage integrase mediated recombinational cloning to rapidly assemble expression elements into targeting vectors followed by phiC31 integrase mediated site-specific integration into a defined hESC chromosomal locus. The platform cell line to which constructs are inserted contains a target locus which has been validated for high transcriptional activity, stability, and resistance to silencing upon differentiation to the three embryonic lineages.

We are working toward optimizing differentiation of pre-engineered hESCs and developing isolation protocols to create high content cell based assays in 'primary' tissues such as neurons, cardiomyocytes, and pancreatic endoderm. These platforms should provide a more relevant and easier-to-use screening system than currently available with rodent models, transformed cells, or primary tissues in culture. Generation of 'platform' hESC lines, stability of expression, ability to accept large, multi-element inserts, and resistance to silencing upon differentiation will be discussed.

Oral Presentation

Microscale Manipulation of Mouse Embryonic Stem Cells for Studying Cell Fusion-Mediated Reprogramming and Diffusible Signaling

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Microsystems have the potential to impact stem cell biology by providing new ways to manipulate cells and the microenvironment around them. We have been developing microsystems that allow us to control the interactions between embryonic stem cells in order to study self-renewal, differentiation, and reprogramming. One of our interests is in studying fusion-induced cell reprogramming. Reprogramming somatic cells to a pluripotent state is one potential route for generating immunologically matched tissues. One approach to reprogramming somatic cells is to fuse them with embryonic stem cells; in some small percentage of the fused cells, factors from the embryonic stem cell are able to reprogram the somatic nucleus. Current approaches to cell fusion, either via polyethylene glycol or electrofusion, do not control pairing of the somatic cells with the embryonic stem cells, and thus typically suffer from low pairing & fusion efficiencies, necessitating lengthy antibiotic selection to select the desired fusions. This selection step prevents researchers from studying early events (<2 days) in reprogramming. Instead, we have developed a PDMS microfluidic device that uses "capture combs" and a three-step back-and-forth loading procedure to pair thousands of cells in parallel. The device is simple to create and use, and we have demonstrated ~70% pairing efficiency and ~70-80% fusion efficiency via electrofusion, which are significant improvements over conventional techniques. By generating a predominantly pure population of fusions, we can avoid the need for antibiotic selection, allowing the study of early events in reprogramming and a better understanding of fusion-mediated reprogramming.

A second activity in our lab is to use microfluidics to modulate diffusible signaling between cells. Obtaining complete information as to which extracellular factors are necessary and sufficient for supporting differentiation or self-renewal of stem cells is critical for understanding stem cell biology. One challenge in standard culture systems is that cell-secreted factors influence the phenotype of surrounding cells in an unknown and uncontrolled fashion, complicating the determination of exactly which factors are required for specific developmental pathways. We have developed arrays of PDMS microfluidic perfusion culture systems to provide a more controlled soluble microenvironment to mouse embryonic stem cells (mESCs). These systems combine culture chambers, valves, debubblers, and fluid interconnect sto provide a complete culture environment. We have found that mESCs prefer to grow and differentiate on tissue-culture polystyrene (versus glass), and thus our perfusion arrays incorporate polystyrene substrates. We have performed experiments and modeling to determine perfusion rates that are high enough to provide sufficient nutrition (and waste removal) yet expose cells to minimal shear. We have also been able to collect and measure cell-secreted LIF from this system, demonstrating the ability to sweep away diffusible factors. Finally, we have been using this system to study both self-renewal of mESCs in the BMP4+LIF defined media system and neuronal differentiation of mESCs in N2B27 defined media. In agreement with suggestions in the literature, our results suggest that these media require cell-secreted factors to function.

Oral Presentation

3D Cell Cultures in Microfluidics for Drug Discovery

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In vitro 3D cell cultures are considered to be more *in vivo* than 2D cell cultures[1], which presently from the majority of the test beds for drug testing. By culturing the cells in a 3D configuration in a microfluidic perfusion system, any drug candidate can be injected. The results can be monitored by analyzing the metabolites, which are collected at the output of the system.

We have developed a 3D microfluidic cell culture system (3D-μFCCS) in a transparent PDMS based system[2]. The system consists of at 1 mm long channel and consists of 2 parts; the cell seeding part and 2 flanking perfusion channels with a width of 200 μm. The cells are captured in a complex coacervation of methylated collagen and

HEMA-MMAMAA while the mixture is injected in to the central inlet. The whole mixture is stabilized by an array of micropillars.

In this fashion we have successfully seeded different cell types, e.g. liver cells (HepG2/C3A), lung cells (L2), fat cells (3T3-L1) and MSC. Viability studies with fluorescence dyes showed these cells were still alive after 72 hours under continuous perfusion.

The 3D-μFCCS can be used as a pre-screening system before testing the drugs on animal models. This has as advantage that it can decrease the cost of drug development up to 10%.

For future applications, this platform can be multiplexed into a more complex system to screen drug metabolism and toxicity, giving the possibility to perform pharmacokinetics studies[3]. An added advantage is that primary human cells can be seeded in the channels which gives a greater impact towards drug validation.

References:

1. Abbott, A., *Nature*, **424**, 870 – 872 (2003)
2. Toh, Y.C., et al., *Lab Chip*, **7**, 302-309 (2007)
3. Viravaidya, K. and M. Shuler, *Biotechnology Progress*, **20**, 590-597 (2004)

KEYNOTE PRESENTATION

Quantitative Systems Analysis of Signaling Networks Governing Cell Phenotypic Behavior

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Cell behavioral functions are controlled by biomolecular networks that translate stimulatory cues (e.g., ligand/receptor binding interactions, mechanical stresses, pathogen infection, environmental insults, etc.) into intracellular signals which regulate transcriptional, metabolic, and cytoskeletal processes that effect proximal and ultimate cell functional responses. While there is a growing body of work enhancing understanding of how intracellular signals are generated by stimulatory cues, an exceptionally difficult challenge at the present time is to understand how these signals operate in integrated manner to govern cell phenotypic behavior. We are addressing this question via a combination of quantitative, dynamic protein-centric experimental manipulations and measurements with a spectrum of computational mining and modeling approaches. Major emphasis is directed toward ascertaining predictive design principles for modulating cell behavior using soluble and/or matrix cues, with complementary endeavor to elucidate logic of interactions among the various molecular components and pathways as an information-processing circuit. This talk will present an overview of our perspective and approach, along with a specific example describing work aimed at understanding integrated operation of signaling pathways governing T-cell behavior.

SESSION 3: SYSTEMS-BASED APPROACHES TO UNDERSTANDING FATE DECISIONS

Invited Presentation

Stem Cell Differentiation: Mechanical Forces, Rhoa and Adhesion Signaling

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In vivo, local tissue structure defines the cellular environment, constraining how cells interact with surrounding extracellular matrix substrates, neighboring cells, soluble growth factors, and physical forces. These "microenvironmental" cues in turn regulate the behavior of individual cells, such as proliferation, differentiation, migration, and suicide. Here, we explore the mechanisms by which cell adhesion to extracellular matrix and neigboring cells regulate the commitment and differentiation of human mesenchymal stem cells to a variety of lineage fates. Adhesion involves the binding and clustering of integrin and cadherin receptors, changes in cell shape and organization, and the generation of active contractile forces against the adhesions. Using microfabrication approaches to engineer extracellular matrix, we show that the degree of adhesion, cell spreading and focal adhesion signaling plays a central role in regulating the commitment of stem cells to osteogenic and adipogenic fates. This adhesion signaling appears to exert its effects by regulating Rho GTPases and the generation of actin cytoskeletal tension. Tension in turn appears to be central to driving the lineage commitment process. Cell-cell adhesions further modulate these mechanical signals leading to more complex patterns of cellular differenitation. These studies demonstrate numerous links between cell adhesion, cell mechanics, and Rho GTPase signaling that are likely important for stem cells to differentiate appropriately in different settings.

Oral Presentation

Engineered Embryonic Stem Cells as a Discovery Tool in Neurobiology: Identification of Molecular Mechanisms of Axonal Degeneration

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In the most prominent neurodegenerative diseases the loss of neuronal processes (axons and dendrites) often precedes the death of the neuronal cell body, thereby causing dysfunction by loss of connectivity. In vertebrates, little is known about molecular mechanisms underlying the elimination of processes, as opposed to those involved in cell body death. We established a novel *in vitro* model allowing the degeneration of neuronal processes to be studied at the biochemical level. The model is based on the controlled over-expression of the neurotrophin receptor p75NTR in highly pure neuronal cultures derived from mouse ES cells. p75NTR is a cell surface glycoprotein re-expressed after lesions of the adult nervous system. Targeting of a p75NTR cDNA into the endogenous tau locus of ES cell-derived neurons causes the synchronous degeneration of their neuronal processes, followed only later by death of the cell bodies. Proteomic analyses performed before visible signs of degeneration led to the identification of the lectin Galectin-1, as a player involved in neuronal process degeneration. Exposure of healthy neurons to this lectin triggers their degeneration, and interfering with Galectin-1 function prevents the degeneration of neurons *in vitro*, as well as in the adult nervous system following excitotoxicity and nerve section. Together, the results illustrate the potential of engineered ES cells as a discovery tool in neurobiology.

Oral Presentation

A Multiparameter Flow Cytometric Assay to Investigate Cytokine Synergies during Erythroid Development

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Synergistic interactions between growth factors underlie developmental processes fundamental to stem cell-based tissue engineering. Despite considerable advances in the development of culture processes for the expansion or differentiation of somatic and embryonic stem cells, a mechanistic understanding of how combinations of growth factors act in concert during stem cell culture is lacking. Stem Cell Factor (SCF) and Erythropoietin (EPO) have a synergistic effect on the production of erythroid cells in cultures of hematopoietic stem cells (HSCs) isolated from peripheral blood, fetal liver, bone marrow and cord blood. Until now, two main mechanisms have been proposed to explain this synergy. First, it is well recognized that SCF and EPO have sequential effects during erythropoiesis; SCF promotes the expansion of primitive hematopoietic progenitor cells while EPO is necessary for later stages of differentiation. A second, more controversial, mechanism implicates the combined and simultaneous effects of SCF and EPO on a cell at the proerythroblast stage. This mechanism has often been referred to as co-signaling or crosstalk downstream of c-Kit and EPO receptor (EPOR). We have developed a serum-free culture system in which SCF and EPO have a significant synergistic effect on the generation of mouse bone marrow erythroid progenitor cells. We are now interested in determining if a specific erythroid cell subset can integrate both SCF and EPO signals. We are using a multiparameter flow cytometric assay to simultaneously detect and quantitate intracellular phospho-proteins (ERK and STAT5) and cell-surface markers (c-Kit, CD-71 and Ter-119) at the single-cell level. The validation experiments for this new assay in primary erythroid cells showed that ERK activation was detected in the c-Kit+ cell subpopulation but not in the c-Kit- cell subset when the cells were stimulated by SCF. Moreover, the effect on the activation of ERK was masked when the whole cell population was analysed. We were also able to determine the kinetics of ERK activation in c-Kit+ cells: the maximum level of activation was reached 5 minutes following cytokine stimulation; after 20 min, the level of phospho-ERK was indistinguishable from the unstimulated cells. At 5 min, the fold change in activation of ERK compared to the unstimulated cells was 2.32 ± 0.47 in c-Kit+ subset, 1.12 ± 0.17 in c-Kit- subset, and 1.14 ± 0.16 in the whole cell population. Measurements of ERK and STAT5 activation in different erythroid cell subsets in response to SCF and/or EPO are underway. Our preliminary results indicate that measurements of kinase activation in specific cell subsets using a multiparameter flow cytometry can provide greater sensitivity than bulk cell assays on heterogeneous populations. This assay will make it possible to efficiently study potential crosstalks between c-Kit and EPO receptor during erythroid development. A better understanding of the mechanisms underlying the synergistic interaction between EPO and SCF in erythropoiesis has the potential to improve the pharmacological treatment of various hematological disorders and to facilitate the large-scale production of erythrocytes for transfusion by delivering cytokines during culture in an optimal fashion.

Oral Presentation

Modeled Stem Cell Niche Assays Using Biomaterial Microarrays

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Using novel Biomaterial Microarrays, we have created an artificial human stem cell niche *in vitro* with characteristics ideal for cultivation and directed differentiation of embryonic stem cells into progenitor cells of hematopoietic lineages. This work has so far resulted in the development of optimal conditions for Stem Cell Biomaterial Microarrays as follows: 1) Poly hydroxyethyl methacrylate coated glass slides. Use of this surface results in inhibition of cell growth in between spots eliminating the possibility of inter-spot cell to cell contact/ cross talk, 2) Poly(D), (L)-lactide/glycolide copolymer (PLGA) as a polymer material spotted on the surface of microarray slides. Non toxic and slow release properties of PLGA (at differing polymer ratios), which is commonly used for tissue engineering and is well documented for delivery of growth factors provides an ideal scaffold for Stem cells on each microarray spot, 3) Oleyl poly (ethylene glycol)-succinyl-N-hydroxy-succinimidyl esters as polymers for immobilization and proper growth of non-adherent cells. This biocompatible anchor for cell membranes provides for efficient attachment, growth and differentiation of non-adherent cells to the surface of the microarrays at the spotted locations. This system is used to test hundreds of culture condition options with and without human mesenchymal stem cells (hMSC) in a very efficient time frame (one experiments provides 9600 options; 96 well plate with 100 "niches" per plate). Additionally, this system allows to quickly screening for the optimal transfection protocols; preliminary experiments with bcl-2 demonstrated high efficiency and stable transfections of human ES cells. The niche is composed of human mesenchymal stem cells, cytokines such as IL-3, IL-6, SCF, TPO, EPO and VEGF, extracellular matrix, etc. The niche is kinetic, e.g. it can release cytokines and tissue factors at different velocity, allowing optimizing growth and differentiation conditions. We will demonstrate the ability to grow human ES cells (NIH code WA01, H1 line) on hMSC, differentiate them into hematopoietic progenitors using TPO and VEGF and enhance erythrocytic differentiation with EPO and DEX. Results of our in-vitro differentiation studies make possible the ultimate goal of our project which is to develop artificial hematopoietic stem cell niche for the future differentiation protocols to develop ES-derived hematopoietic progenitors.

Our goal is to develop models whose behavior are realistically simple with respect to the details of the interactions and yet produce the desired global behavior. Such models, while often not so precise in detailed quantitative predictions, will be able to yield experimentally observed patterns of behavior, such as how agents interact and cooperate under given circumstances, often difficult to obtain *in vivo*. It will be much easier to run what-if experiments than to conduct real system experiments. With a well-designed model, theoretical reasoning can be built about stem cell proliferation, differentiation, stem cell-niche interaction etc. In silico simulations thus have a lot to contribute as powerful research tools in unraveling stem cell behavior and more so in case of complex phenomena like transplantation.

We will present results of several of our simulations. We will discuss in depth a simple model to understand what mechanism might be employed for maintaining the number of stem cells in the bone marrow and producing a continuous output of differentiated cells. This model provides an example of how a simulation based on a simple mathematical model can show what properties of stem cells might be required to enable the maintenance of the systems homeostasis. The niche is modeled as having the ability to maintain a reasonably fixed number of stem cells, produce supply of mature (differentiated) cells, and to be capable of recovering to this state even after a major deviation resulting from an injury or disease. The cell behavior is determined by both internal (intrinsic) factors and external (extrinsic) factors.

SESSION 4: INTERCELLULAR SIGNALING AND THE ENGINEERED NICHE

Invited Presentation

Spatial Control of Auto-Regulatory Signaling to Modulate Embryonic Stem Cell Self-Renewal and Differentiation

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Local micro-environmental cues consisting of soluble cytokines, extra-cellular matrix, and cell-cell contacts are determining factors in stem cell fate. These extrinsic cues form a 'niche' that governs a stem cell's decision to either self-renew or to differentiate into one or more cell types. We have recently demonstrated that mouse embryonic stem cells (ESC) form an autoregulatory niche1, where in the rate of differentiation is modulated by a feed-forward loop involving LIF and JAK/STAT3 signaling[2,3]. Using micropatterning to contro[l] ESC colony size we have recently demonstrated the applicability of these concepts in regulating signaling gradients and fate in human ESC[4]. Mechanistically, a size-dependent spatial gradient of Smad1 signaling is generated as a result of antagonistic interactions between hESCs and extra-embryonic endoderm (ExE). These interactions are mediated by the secretion of bone morphogenetic protein-2 (BMP2) by ExE, and its antagonist growth differentiation factor-3 (GDF3) by hESCs, into spatially-restricted microenvironments. Extending these observations for the scalable generation of targeted cell types in bioreactors requires modulating endogenous and exogenous signaling in three-dimensions. Our recent results on our use of new technologies to create and seed ESC aggregates into bioreactors, and on how the size and composition of these aggregates can be controlled to guide cell fate, will be reviewed. Together these studies demonstrate how stem cell fate can be controlled using spatial control of cell-cell interactions.

References:

1. Davey et al., *Stem Cells*, **24**(11): 2538-48 (2006)
2. Davey et al., *FASEB J*, **21**(9):2020-32 (2007)
3. Mahdavi et al., *PLOS Computational Biology*, **3**(7): 1-11(2007)
4. Peerani et al., *EMBO J.*, **26**(22): 4744-55 (2007)

Oral Presentation

Engineering Intercellular Signals of the Thymic Niche: Towards Generation of Functional T Cells from Stem Cells

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T-cells develop from hematopoietic progenitor cells (HPCs) through a series of complex molecular interactions in the thymus. Although a variety of environmentally controlled events take place in the thymus, research has elucidated several key intercellular signaling events that are responsible for the development of mature T cells: the notch-signaling pathway and the TCR/MHC signaling process. It is now established that notch-delta ligand signaling between thymic stromal cells and HPCs is essential to T lineage commitment while efficient positive and negative selection requires engagement of TCR and either Class I or Class II MHC molecules expressed by epithelial cells and macrophages. Our goal is to quantitatively mimic this complex thymic cell signaling niche using notch ligand and tetramer functionalized microbeads i.e. materials that can act as artificial stromal cells and artificial thymic epithelial cells. Specifically we are using the notch ligand DLL4 and a Class MHC I tetramer loaded with an LCMV antigenic peptide. We hypothesize that in the presence of appropriate soluble factors, HPCs can be efficiently directed to mature, antigen-specific, functional T cells by using such controlled, bead-based cell signaling. Our results indicate that bone marrow derived cKit+Sca1+ HPCs are efficiently differentiated into early T cells using DLL4 signaling and that intracellular notch signaling can be quantitatively controlled using this system. We have also demonstrated, for the first time, that early T cells (e.g. CD4+CD8+ cells) can be directed to generate antigen specific, cytotoxic CD8+ T cells using antigen-loaded MHC Class I tetramers. Further studies are currently underway to quantitatively study the effects of ligand density on T cell differentiation and to elucidate the mechanism of T cell maturation.

Oral Presentation

Embryoid Body Shell Formation Reduces Diffusive Transport of Inductive Biochemicals

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Embryoid bodies (EBs) recaptilulate the early stages of embryonic development and are therefore a unique system for differentiation of human embryonic stem (hES) cells into therapeutically-relevant cells. In this system, hES cells aggregate as dense three-dimensional clusters which transform into larger cystic bodies[1]. Differentiation of cells towards specific lineages is often assisted by treating the EBs with soluble biochemicals such as cytokines, growth factors and vitamins. These exogenous biochemicals are added as supplements to the media; thus inductive activity is dependent on diffusive transport into the EB. We investigated how diffusive transport of biochemicals is influenced by structural changes in natural EB development. A scanning electron microscopy analysis, conducted over 14 days, revealed time-dependent changes in EB surface structure which led to the formation of a 20 μm shell that significantly reduced the diffusive transport of a model molecule (374 Da) by >80%. We found that the shell consists of a cellular network interwoven with extracellular matrix material, of which collagen is identified as a component. Disrupting the shell promoted increased effective diffusion. Diffusive transport was restored to more than 40% after a mild collagen digestion. Our results suggest that limitations in the diffusive transport of biochemicals need to be considered when formulating EB differentiation strategies. A reduction in diffusive transport to the cell may trigger the activation of mitochondrial apoptotic inducing factor (AIF), which has been shown to be a pathway for programmed cell death[2] during embryonic morphogenesis. The development of hemangioblasts, progenitor cells with both hematopoietic and vascular potential, in the EB[3] on days 3-4 coincides with EB shell formation; hence reduced diffusive transport may be a mechanism for stem cell differentiation.

References:

1. Itskovitz-Eldor, J. et al., *Molecular Medicine*, **6**:88 (2000).
2. Joza, N. et al., *Nature*, **410**:549 (2001).
3. Kennedy, M., S. L. D'Souza, M. Lynch-Kattman, S. Schwantz, and G. Keller, *Blood*, **109**: 2679 (2007).

Oral Presentation

Role of Adhesion Molecules in Hematopoietic and Endothelial Commitment of Murine Embryonic Stem Cells

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Mouse embryonic stem cells (ESC) collect information from their environment and make cell fate decisions based on intrinsic and extrinsic factors. Embryoid body (EB) formation induces the differentiation of ESC to multiple tissue lineages. Cell-cell contact or cell-environment interactions influence EB formation and ESC fate decisions within EB. However, the molecular mechanisms underlying modulation of ESC fate decisions by cell-environmental interactions are incompletely understood. Adhesion molecules influence proliferation and differentiation in multiple developing and adult tissues. We hypothesize that adhesion molecule interactions have a critical role in guiding ESC commitment to hematopoietic and endothelial lineages. Quantitative RT-PCR was used to establish the relative levels of adhesion molecule expression during EB formation and early stages of hematopoietic differentiation. Relative expression profiles were generated for 32 adhesion molecules at discrete stages of early hematopoietic and endothelial development. Adhesion molecules differentially expressed under these conditions were primarily representative of adherens junction, tight junction and gap junction pathways. These genes include E-cadherin, claudin-4, connexin-43, connexin-45, zona occludens-1 (ZO-1) and zona occludens-2 (ZO-2). Differential regulation of molecules in the junction pathways hematopoietic and endothelial development supports the hypothesis that cell-cell interactions are important for ESC fate decisions. Stable ESC lines constitutively knocking down expression of E-cadherin, Connexin-43 and ZO-1 were generated using lentiviral transductions of shRNA constructs. Expression of E-cadherin, Connexin-43 and ZO-1 are dispensable for the formation of early hematopoietic and endothelial cells which express Fetal Liver Kinase-1 (Flk-1). Expression of CD45, an extracellular protein found on most hematopoietic cells, is slightly decreased in cell lines decreased in expression of E-cadherin and ZO-1. A parallel increase in endothelial differentiation is observed in these knockdown lines in endothelial sprouting assays. Functional and molecular assays for hematopoietic and endothelial commitment are ongoing to determine the consequence of manipulation of adhesion molecule expression levels on transitional states downstream of Flk-1 expression.

Oral Presentation

Activin Alters the Kinetics of Endoderm Induction in Embryonic Stem Cells Cultured on Collagen Gels

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Embryonic stem cell-derived endoderm is critical for the development of cellular therapies for the treatment of disease such as diabetes, liver cirrhosis, or pulmonary emphysema. Here, we describe a novel approach to induce endoderm from mouse embryonic stem cells (mES) using fibronectin-coated collagen gels. This technique results in a homogenous endoderm-like cell population, demonstrating endoderm-specific gene and protein expression, which remains committed following *in vivo* transplantation. In this system, activin, normally an endoderm inducer caused an 80% decrease in the Foxa2 positive endoderm fraction, while follistatin increased the Foxa2 positive endoderm fraction to 78%. Our work suggests that activin delays the induction of endoderm through it transient precursors, the epiblast and mesendoderm. Long term differentiation, displays a two-fold reduction in hepatic gene expression and three-fold reduction in hepatic protein expression of activin-treated cells compared to follistatin-treated cells. Moreover, subcutaneous transplantation of activin-treated cells in a syngeneic mouse generated a heterogeneous teratoma-like mass, suggesting these were a more primitive population. In contrast, follistatin-treated cells resulted in an encapsulated epithelial-like mass, suggesting these cells remained committed to the endoderm lineage. In conclusion, we demonstrate a novel technique to induce the direct differentiation of endoderm from mES cells without cell sorting. In addition, our work suggests a new role for activin in induction of the precursors to endoderm, and a new endoderm-enrichment technique using follistatin.

SESSION 5: NOVEL APPROACHES FOR EMBRYONIC STEM CELL GROWTH AND DIFFERENTIATION

Invited Presentation

Controlling Human Embryonic Stem Cells by Niche Regulation

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It is becoming increasingly evident that the distinct properties that define stem cells are not autonomously achieved and require external require from their microenvironment, termed the stem cell niche. Although the stem cell niche has been characterized *in vivo*, there is little evidence to suggest the niche plays a regulatory component in stem cells derived and propagated *in vitro* such as human pluripotent stem cells (embryonic or induced from fibroblasts). Using a proteomic strategy, we have recently identified the assemblance of a niche relationship among heterogeneous cells that allow the survival and growth of human embryonic stem cell lines. Although there are several components to this niche, both at the level of extracellular matrix and soluble factors, most importantly there is a delineation between the pluripotent cells and niche cells that sustain the most primitive stem cell types *in vitro*. Our original observations and additional findings will be discussed on how the niche regulates self renewal of primitive insulin growth factor receptor expressing pluripotent stem cells and how this niche can be further characterized to potentiate and, perhaps, control lineage specification. These latter observations suggest the niche is not only regulatory to self renewal divisions of human pluripotent stem cells, but also continues its regulatory function during lineage specification in differentiating embryoid bodies.

Oral Presentation

Engineering Embryonic Stem Cells to Create a Cell Therapy for Diabetes

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Of paramount importance for the development of cell therapies to treat diabetes is the production of sufficient numbers of pancreatic endocrine cells that function similarly to primary islets. The very large numbers of cells needed to treat human patients will necessitate a directed differentiation paradigm in which most or all of the lineage decisions between an ES cell and the beta-cell are guided efficiently. We have developed an *in vitro* differentiation process that converts human ES cells to endocrine cells capable of synthesizing the pancreatic hormones insulin, glucagon, somatostatin, pancreatic polypeptide, and ghrelin. This process resembles *in vivo* pancreatic organogenesis by directing cells through stages resembling definitive endoderm, gut tube endoderm, pancreatic endoderm, and endocrine precursor cells, which then differentiate into to endocrine hormone-expressing cells. The hES cell-derived insulin-expressing cells have insulin content approaching that of adult pancreatic islets; and similar to fetal beta cells, they release C-peptide in response to multiple secretory stimuli, but only minimally in response to elevated glucose. Transplantation of the *in vitro* hESC-derived cells into immunocompromised mice resulted in stable engraftment and detectable human C-peptide in the blood.

Oral Presentation

Differentiation of Murine Embryonic Stem Cells to Cardiomyocytes Is Increased Under Reduced Oxygen

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Introduction: Cardiomyocytes derived from differentiated embryonic stem (ES) cells hold promise as a treatment for heart disease, but generation of sufficient quantities of differentiated cells remains a challenge. Most ES cell research is performed in incubators with a humidified 95% air/5% CO_2 gas mixture, resulting in a gas-phase oxygen partial pressure (pO_2gas) of 142 mmHg. Embryonic cells in early development are exposed to pO_2cell values of 0-30 mmHg, and the effects of such conditions on differentiating ES cells are poorly understood. Here we show that control of the pO_2cell to levels experienced by the developing embryo enhances differentiation of ES cells into cardiomyocytes.

Methods: Embryoid bodies (EBs) were formed in hanging drops containing 500 ES cells in 20 µl of DMEM supplemented with 10% FBS and ascorbic acid. After 2 days, EBs were transferred to culture dishes fabricated with a highly oxygen permeable, fibronectin-coated silicone rubber membrane on the bottom, to which the cells attached and grew. Three days later, the medium was changed to a serum-free ITS medium with ascorbic acid and grown 5-6 more days with daily medium changes. pO_2gas was controlled by placing culture dishes in airtight containers purged with premixed gas containing 5% CO_2 and either 142, 36, or 7 mmHg oxygen. By using silicone rubber membrane-based dishes for all conditions, precise control of pO_2cell at the cell-membrane interface was achieved. Cardiomyocytes were identified by flow cytometry of cells immunostained with an antibody to sarcomeric myosin heavy chain (MF-20) and confirmed with MF-20 and anti-cardiac troponin T (cTnT) immunostaining of histological sections and real-time RT-PCR quantification of cardiac markers (Nkx2.5, cTnT, and Mef2c).

Results: After differentiation of ES cells at different, constant pO_2gas conditions for 11 days, the fraction of cells that were cardiomyocytes, assessed by flow cytometry, averaged 29%, 20%, and 9% at a pO_2gas of 7, 36 and 142 mmHg, respectively. The number of cells was about the same at 142 and 36 mmHg and smaller at 7 mmHg. Consequently, the number of cardiomyocytes was similar at 7 and 142 mmHg but significantly higher at 36 mmHg (58 cardiomyocytes per original ES cell). The number fraction of MF-20 positive cells by immunostaining tissue sections was 45%, 20%, and 5% for samples taken from tissue cultured at 7, 36, and 142 mmHg, respectively. Cells cultured for 11 days at 7 mmHg preferentially formed thin cell sheets and smaller aggregates than were found at 36 or 142 mmHg.. Culture of differentiating cells at pO_2gas of 142 and 7 mmHg oxygen for different time periods in different orders for 10 days resulted in the fraction and number of cardiomyocytes always being greater when cells were initially at 7 compared to 142 mmHg oxygen; the fraction increased with time cultured at 7 mmHg oxygen up to 6 days, with little change thereafter. Differentiation for 6 days at 36 mmHg followed by 15 days at 142 mmHg gave the best results. In a single series of experiments, the maximum cardiomyocyte fraction, 57%, was 5 times larger, and the number of cardiomyocytes was 9 times larger, than for constant culture at 142 mmHg oxygen, and 304 cardiomyocytes were generated for each initial ES cell. Calculations with a model of oxygen consumption and diffusion in the aggregates suggest that enhanced differentiation occurs for pO_2cell in the range of 1 to several 10s of mmHg. Low oxygen differentiation resulted in increased expression of Nkx2.5, cTnT, and Mef2c compared to culture under constant 142 mmHg oxygen at day 10 but not by day 5.

Conclusions: These results demonstrate that culture at low pO_2cell markedly increases differentiation of ES cells into cardiomyocytes. This finding may enhance prospects for improving productivity and yield of cardiomyocytes, thereby enabling their therapeutic use in heart disease. These results also suggest that low oxygen may be important during embryonic development and possibly affects the differentiation of ES cells to other cell types.

Oral Presentation

Molecular Imaging of Cardiac Constructs Derived from Human Embryonic Stem Cells

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Molecular imaging is a documented modality for monitoring the *in vivo* kinetics of stem cell injections. The application of this platform to track the survival of stem cell-based myocardial grafts is a logical step in advancing the field of therapeutic tissue engineering. In the present study, we seek to answer fundamental questions of stem cell-derived cardiac therapeutics using tissue engineering, dynamic conditioning and molecular imaging.

Engineered myocardial tissue (EMT) was formed by suspending H9 human embryonic stem cell-derived cardiac myocyte precursors (hESC-CMs) transfected with a lentiviral vector carrying a double-fusion reporter gene consisting of firefly luciferase and green fluorescent protein (Fluc-GFP) in a three dimensional extracellular

matrix. Ex vivo bioluminescence imaging (BLI) was used to evaluate EMT viability after 7 days of mechanical conditioning. Cellular morphology and the expression of cardiac proteins were evaluated after 10 days in culture, and the effect of conditioning was also assessed. Constructs were transplanted subcutaneously in the leg muscle of SCID mice (n = 6) and monitored using BLI. All constructs were explanted at 8 weeks and assessed with H&E staining and immunohistochemistry.

This study reports that *in vitro* mechanical conditioning promoted aligned cellular morphology and increased expression of the cardiac protein, troponin I over static controls. BLI signals demonstrated *in vivo* viability over a period of 8 weeks, and anti-GFP positive cells corresponded with the signal location; however, histological analysis revealed that EMT was unable to maintain a cardiac phenotype *in vivo* over the length of the study. Future directions of study include quantitative assays of cardiac phenotype in engineered tissues and monitoring commitment to a cardiac phenotype following transplantation.

Oral Presentation

The Effect of Controlled Growth Factor Delivery on Embryonic Stem Cell Differentiation Inside of Fibrin Scaffolds

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The goal of this project is to develop 3-D biomaterial scaffolds that present cues to direct differentiation of embryonic stem cell derived neural progenitor cells (ESNPCs)seeded inside into mature neural phenotypes, specifically neurons and oligodendrocytes. Release studies were performed to determine the appropriate conditions for retention of neurotrophin-3 (NT-3), sonic hedgehog (Shh), and platelet derived growth factor (PDGF) by an affinity-based delivery system (ABDS) incorporated into fibrin scaffolds. Embryoid bodies (EBs) containing neural progenitors were formed from mouse ES cells, using a 4-/4+ retinoic acid treatment protocol, and then seeded inside of fibrin scaffolds containing the drug delivery system. This delivery system was used to deliver various growth factor doses and combinations to the cells seeded inside of the scaffolds. Controlled delivery of NT-3 and PDGF simultaneously increased the fraction of neural progenitors, neurons, and oligodendrocytes while decreasing the fraction of astrocytes obtained compared to controls containing no growth factors in the media. These results demonstrate that such a strategy can be used to generate an engineered tissue for the potential treatment of spinal cord injury and could be extended to study of differentiation in other tissues.

SESSION 6: NOVEL APPROACHES FOR ADULT STEM CELL GROWTH AND DIFFERENTIATION

Invited Presentation

Neurogenesis in the Adult Mammalian Brain

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While most neurons in the adult central nervous system (CNS) are terminally differentiated, evidence now exists that small populations of neurons are generated in the adult olfactory bulb and hippocampus. In the adult hippocampus, newly born neurons originate from putative stem cells that exist in the subgranular zone of the dentate gyrus. Progeny of these putative stem cells differentiate into neurons in the granular layer within a month of the cells' birth, and this late neurogenesis continues throughout the adult life of all mammals. Stem cells can be harvested from a variety of brain and spinal cord regions, genetically modified, and transplanted back to the brain and spinal cord where they can differentiate into mature glia and neurons depending on the local environment. In addition, environmental stimulation can differentially affect the proliferation, migration, and differentiation of these cells *in vivo*. These environmentally induced changes in the structural organization of the hippocampus, result in changes in electrophysiological responses in the hippocampus, as well as in hippocampal related behaviors. We are studying the cellular, molecular, as well as environmental influences that regulate neurogenesis in the adult brain and spinal cord. We have recently identified several novel mechanisms that regulate proliferation, survival and differentiation of these adult derived stem cells. The functional and practical significance of these findings will be discussed in light of their implications for alternative or expanded views of structural plasticity in the adult brain.

Oral Presentation

Engineering Stromal Cells for Supportive Differentiation of TCR-Transduced Hematopoietic Stem Cells into CD4 T Cells *In Vitro*

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Development of novel methods for generation of TCR (T cell receptor)-engineered T cells may have clinical applications for immunotherapy against tumors and infections. It was shown that the mouse bone stromal cell line OP9 expressing a notch ligand (Delta-like-1, DL1) can support the differentiation of hematopoietic stem cells into cytotoxic CD8 T cells *in vitro*. We tested a hypothesis that introduction of I-Ab, a mouse MHC class II molecule, into the OP9-DL1 cells could make them facilitate the differentiation of hematopoietic stem cells into helper CD4 T cells. We found that hematopoietic stem cells transduced to express a CD4 TCR could develop into functional CD4 T cells when cultured on OP9-DL1, but expression of I-Ab could significantly improve not only the development but also the functionality of these T cells, measured by their ability to secrete inflammatory cytokines. This finding can have broad implications for engineering hematopoietic stem cells to be functional T cells.

Oral Presentation

Understanding Bio-Chemical Changes in the Aged Muscle Niche

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The age-specific lack of tissue repair which leads to degeneration and loss of function is perfectly exemplified in skeletal muscle, and we are attempting to understand this aging process in cellular and

molecular terms. In this regard, we have identified the key biochemical pathways that function in young muscle stem cells for tissue maintenance and repair and that become deregulated with age, causing poor regeneration and decreased function of skeletal muscle. Forced manipulation of these signaling networks yields control over muscle stem cell responses, enables novel methods for manufacturing ex-vivo self-assembling muscle fibers that are capable of continuous regeneration and enables new approaches for rejuvenating the capacity for tissue repair. Importantly, the tissue produced by the "aged" stem cells appears to be relatively "young" with respect to telomerase activity and telomere length. These findings suggest novel strategies for improving muscle regeneration and combating loss of functional tissue caused by the aging process or by the degenerative disorders.

Oral Presentation

Directed Differentiation of Multipotent Adult Progenitor Cells into the Cells of the Hepatic Lineage

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Several stem or progenitor cells from the bone marrow, fetal and adult liver and embryonic stem cells are currently being evaluated for their potential to differentiate into the cells of hepatic lineage efficiently. In addition to their potential use in cellular therapies, other applications include their use in drug toxicity studies, bioartificial liver support, tissue engineering, and as a model system to study development and disease. Multipotent adult progenitor cells (MAPCs) isolated from postnatal rat, mouse, and human bone marrow can be expanded *in vitro* without senescence and can differentiate *in vitro* or *in vivo*, into different cell types of the three germ layer lineages. We have recently developed a four-step 21-day differentiation protocol optimized for medium components, oxygen levels and extra-cellular matrix for efficient differentiation to cells with morphological, phenotypic, and functional characteristics of hepatocytes from MAPCs. We observe the time dependent expression of early endodermal genes, including Goosecoid, Cxcr4 and Hnf3b representing the progression through definitive endoderm, followed by Afp and transthyretin (Ttr) corresponding to the onset of hepatic specification, and expression of albumin, glucose 6 phosphatase (G6p), and cytochrome P450 depicting maturation at levels expressed in fetal liver by the end of differentiation. In addition to gene expression, the differentiated cells expressed Hnf3b, Afp, Ck18 and albumin at the protein level and their functional nature was evaluated by albumin ELISA and PAS staining for glycogen storage. Similarly, we also have evidence for the applicability of this protocol for differentiation of human embryonic stem cells to the hepatic lineage. In addition to differentiation of MAPCs in 2D monolayer, we have also investigated the ability of MAPCs to self-assemble into 3D aggregates and explored the possibility of enhanced differentiation. MAPCs were successfully induced into 3D aggregates that exhibited good viability, morphology, and undifferentiated phenotype in terms of expression of high levels of Oct4 and lack of expression of differentiated markers when formed under MAPC media and 5% oxygen. The aggregates retained the ability to undergo spontaneous multi-lineage differentiation as well as directed differentiation to the hepatic lineage with improved expression of Hnf4a, a transcription factor, DPPIV, a bile duct protein, and Cyp2b1 and G6p, functional hepatic markers in 3D compared to their expression in corresponding 2D differentiation. Other than the advantage of obtaining more functionally mature differentiated cells, 3D culture provides a unique model system for studying nascent 3D development and can potentially help in the design of scalable culture systems that can be monitored and controlled to enhance differentiation.

Oral Presentation

Maximization of the Ex-Vivo Expansion of Human Hematopoietic Stem/progenitor Cells by Direct Contact Culture with Mesenchymal Stem Cells

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The ex-vivo expansion of hematopoietic stem/progenitor cells (HSPC) is highly desirable in a variety of clinical settings ranging from transplantation to gene therapy. In particular, if successful expansion protocols were established, the difficulties associated with inadequate numbers of HSPC collected for transplantation would be largely eliminated, especially for umbilical cord blood (UCB), which contains a limited number of progenitors.

We evaluated the role of human mesenchymal stem cells (MSC) from the adult bone marrow (BM) in supporting the ex-vivo expansion/maintenance of human HSPC from either BM or UCB. CD34+ enriched cells were cultured in serum-free medium supplemented with SCF, bFGF, LIF and Flt-3L, in the presence/absence of MSC, and analyzed for proliferation, phenotype and clonogenic potential. In terms of total cells, maximal levels of expansion, obtained for BM cultures, were much higher in the presence of MSC (260 versus 32 in feeder free conditions, respectively); for UCB cells, we could not efficiently expand the cells in the absence of MSC (maximal fold increase of 0-36), whereas when the feeder cells were present, the maximal levels of expansion ranged 441. MSC were also efficient in supporting the expansion of the more primitive progenitors (CD34+ and CD34+CD38- cells) from both BM and CB. The differentiative potential of both BM and CB CD34+ enriched cells co-cultured with MSC was primarily shifted towards the myeloid lineage, while maintaining/expanding an early lymphoid CD7+ population. Clonogenic analysis of the expanded cells also showed an increase in progenitors of the myeloid lineage. These results indicate that BM MSC support an efficient expansion/maintenance of HSPC from both BM and UCB.

Since the nature of the hematopoietic supportive capacity of MSC remains unsolved especially concerning the dependency on the direct contact between MSC and HSPC, next we cultured both BM and UCB HSPC: (i) directly in contact with the feeder layer of MSC (Contact); (ii) separated from MSC by a microporous membrane of 0.4 micrometer (Non-contact); or (iii) in the absence of MSC.

Total cell expansion was much higher in a Contact configuration for both BM and UCB cells, compared to Non-contact configuration: average maximal fold increases at day 18 of 260 (BM) and 441 (UCB) versus 30 (BM) and 117 (UCB) for Contact and Non-contact, respectively, attesting the dependency on direct cell contact to achieve a more effective expansion of HSPC. The maintenance of the stem/progenitor content was clearly favoured by the direct contact with MSC, especially early in culture. Later on, in all configurations, for both BM and

UCB cells, the few percentage of CD34+ progenitors at day 18 coincides with the fraction of the more primitive CD34+CD38- cells. In addition, we were not able to maintain/expand a CD7+ population in the absence of MSC. However, since this population is expanded/maintained in Non-contact we hypothesized that MSC act through a soluble activity on this phenotype expression by hematopoietic cells, rather than through cell-to-cell contact only.

These results attest the feasibility of using human BM MSC to support HSPC from both BM and UCB, while revealing the importance of direct contact between HSPC and MSC, especially for UCB, to maximize HSPC expansion *in vitro* to obtain a therapeutically significant amount of cells from the available donor samples, with implications in terms of bioreactor design.

KEYNOTE PRESENTATION

Normal and Neoplastic Stem Cells

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Following embryonic development, most of our tissues and organs are continuously regenerated from tissue/organ specific stem cells. The principal property that distinguishes such stem cells from their daughter cells is self-renewal; when stem cells divide they give rise to stem cells (by self-renewal) and progenitors (by differentiation). In most tissues only the primitive stem cells self-renew. Stem cell isolation and transplantation is the basis for regenerative medicine. Self-renewal is dangerous, and therefore strictly regulated. Poorly regulated self-renewal can lead to the genesis of cancer stem cells, the only self-renewing cells in the cancerous tumor.

SESSION 7: BIOMATERIALS FOR STEM CELL GROWTH AND DIFFERENTIATION

Invited Presentation

Matrix Elasticity Directs Stem Cell Lineage Specification

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Microenvironments appear important in stem cell lineage specification but can be difficult to adequately characterize or control with soft tissues. Naive mesenchymal stem cells (MSCs)are shown here to specify lineage and commit to phenotypes with extreme sensitivity to tissue level elasticity. Soft matrices that mimic brain are neurogenic, stiffer matrices that mimic muscle are myogenic, and comparatively rigid matrices that mimic collagenous bone prove osteogenic. The latter also mimic highly fibrotic environments such as the post-infarct scare that has recently been shown to induce osteogenesis of MSCs in mice [Breitbach Blood 2007]. In culture, during the initial week, reprogramming of the three lineages is possible with addition of soluble induction factors, but after several weeks in culture, the cells commit to the lineage specified by matrix elasticity, consistent with the elasticity-insensitive commitment of differentiated cell types. Inhibition of nonmuscle myosin II blocks all elasticity directed lineage specification – without strongly perturbing many other aspects of cell function and shape. The results have significant implications for understanding physical effects of the *in vivo* microenvironment and also for therapeutic uses of stem cells.

Oral Presentation

Biomimetic Materials to Control Human Embryonic Stem Cell Self-Renewal and Fate Determination

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Human embryonic stem cells (hESCs) are being studied as potential source of cells for the treatment for many diseases (e.g. diabetes, Parkinson's, leukemia). The successful integration of hESC into such therapies will hinge upon three critical steps: stem cell expansion in number without differentiating (i.e., self-renewal); differentiation into a specific cell type or collection of cell types; and, promotion of their functional integration into existing tissue. Precisely controlling each of these steps is essential to maximize hESC's therapeutic efficacy, as well as to minimize potential side effects that can occur when the cells numbers and types are not properly controlled. However, it is difficult to precisely control the behavior of hESCs, since environmental conditions for self-renewal and differentiation are incompletely understood. Currently, hESCs are typically grown on a feeder layer of mouse cells (i.e., irradiated but viable cells) and/or conditioned with media derived from these cells, and would therefore be considered xenografts if implanted into humans. Furthermore, current hES cell lines are also "contaminated" by foreign oligosaccharide residues picked up from mouse feeder cells and culture medium, and therefore have limited clinical potential. Although newer hES cell lines have been derived on human feeder layers, this system suffers from poor reproducibility and has limits for large-scale hESC expansion.

Control of hESC self-renewal and differentiation within a synthetic system offers several advantages. If hESCs can be derived and maintained within a completely synthetic environment, then it will be possible to eliminate pathogen transmission associated with mouse or human feeder layers, provide a scalable basis for large-scale production of hESCs, and provide precise methodology for controlling hES cell differentiation.

My group is working on developing a novel platform technology consisting of a completely synthetic environment to precisely control hES self-renewal and subsequent differentiation into a defined phenotype. To achieve the purported goals of regenerative medicine, methods for the precise control of the survival, proliferation, and differentiation of stem cell populations *in vitro* and *in vivo* are necessary. We aim to engineer a tunable and well-defined environment presenting a completely synthetic extracellular matrix, chemically defined media, and environmental conditions (e.g., media pH & pO2, bioreactor physical conditions, etc…). The result will be a technology platform that can be applied to numerous stem cell populations and understand self-renewal as a biological/developmental phenomenon.

This presentation will focus on the following three main themes related to creating synthetic environments to control of hESC self-renewal and differentiation: 1) control of hES cell interaction with synthetic biomimetic matrices [i.e., mechanically and chemically tunable materials]; 2) development of chemically defined hESC culture media; and, 3) fate determination from chemically and mechanically defined environmental conditions. By emphasizing these themes, we can create biomaterials, synthetic media, and cell culture protocols that can increase the reproducibility of culture conditions and elucidate the requirements for hESC maintenance and differen-

tiation. This approach will also yield novel insights into environmental regulation of hES cell biology, provide novel systems for *in vitro* hES expansion, and create methods that enhance control of transplanted stem cell populations to develop into specific tissues.

Oral Presentation

3D Microwell Array Culture of Human Embryonic Stem Cells Reveals Effects of Colony Morphology on Cell Growth and Differentiation

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Human embryonic stem cells (hESCs) require cell-cell contact to survive. In addition, intercellular signaling through direct contacts is an important factor in differentiation of many cell types, including neural, cardiac, pancreatic islet, and epithelial cells, and is likely involved in differentiation of hESCs to cells in these lineages. Typical hESC culture methods generate colonies with varying sizes and shapes, which in turn give rise to embryoid body (EB) populations that possess a very wide size distribution. In an attempt to improve heterogeneity of hESC colony and EB size and shape, and to potentially enhance self-renewal and differentiation efficiency, we engineered microwell surfaces that confine growing hESC colonies to desired sizes and shapes. Wells are molded in a polyurethane substrate using a polydimethylsiloxane (PDMS) stamp. Gold is then deposited on the areas between the wells and a self-assembled monolayer of a triethylene glycol (EG3) terminated alkanethiols is formed on the gold to repel protein and cell attachment. Extracellular matrix proteins are adsorbed inside the wells and cells are seeded into the wells. Over the course of several days to weeks the cells divide and colonies fill the wells, generating a uniform population of colonies with identical morphologies. The colonies can be harvested intact by enzymatic dissociation of the extracellular matrix to yield a monodisperse population of EBs. Maintaining colonies at a diameter of 200 microns or smaller inhibits spontaneous differentiation in the presence of the appropriate self-renewal factors. In addition, we observed effects of EB size on differentiation potential, with small EBs favoring ectodermal differentiation and larger sizes favoring mesodermal lineages. Microwell culture of colonies with defined, heterogenous morphologies at a high density has potential applications in culture scaleup, directed differentiation, and high throughput screening applications.

Oral Presentation

Microparticle Delivery of Morphogenic Factors within Embryoid Bodies for Directed Embryonic Stem Cell Differentiation

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Pluripotent embryonic stem cells (ESCs) represent a potentially robust cell source for the treatment of various degenerative cellular diseases, however the inability to efficiently direct differentiation of the cells limits their potential clinical utility for regenerative therapies. Differentiation of ESCs *in vitro* is commonly induced via 3D cell aggregates, commonly referred to as "embryoid bodies" (EBs) which yield a heterogeneous population of different cell types comprising the three germ lineages (ectoderm, endoderm and mesoderm). Incorporation of degradable polymeric microparticles directly within EBs may enable locally controlled spatiotemporal presentation of morphogenic factors to ESCs in a manner capable of enhancing directed differentiation of the cells. Our results to date demonstrate that microparticle incorporation within EBs can be controlled by combinations of different adhesive protein coatings, microparticle to cell feed ratios and dynamic mixing conditions. Using this approach, controlled release of differentiation factors, such as retinoic acid, from PLGA microparticles enhances the efficiency and purity of ESC differentiation, compared to soluble delivery methods. These results indicate that biomaterials based approaches to regulate stem cell microenvironments may significantly improve the efficiency of directed differentiation methods.

Oral Presentation

Arrayed Cellular Microenvironments for Exploring Cell Fate

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The cellular microenvironment is crucial in determining cellular behavior. This microenvironment, composed mainly of extracellular matrix proteins (ECMPs), growth factors (GFs), glycans (GCs), and mechanical forces, behaves in a complex manner to affect cell fate. Experimental manipulation of these environments to affect proliferation and differentiation of human embryonic stem cells (hESCs) is central to developing strategies for the production of defined cell types to treat a variety of disorders in which cells are defective, damaged or dead.

Current technologies are inadequate to screen the vast number of combinations of factors that may influence cell fate. Previously, we described an array technology that we developed to probe the interactions of several ECM components on maintenance of primary rate hepatocyte phenotype and differentiation of mouse embryonic stem cells (mES) towards an early hepatic fate (Flaim et al., Nature Med. 2:119-125, 2005). We have expanded this technology by 1) incorporating active GFs and GCs into the arrayed microenvironments and 2) expanding the image acquisition and analysis for the real time simultaneous screening of thousands of physiochemical parameters on cellular attachment, proliferation, and gene and protein expression. This novel approach allows for the systematic assessment and probing of the complex relationships between various cell types and their microenvironment.

The microenvironmental components that promote hESC self-renewal are unknown. Although, hESCs can be expanded and maintained in a pluripotent state either in a co-culture with mouse embryonic fibroblasts (MEFs) or with the assistance of commercially available substrates, defined conditions for culturing hESCs have not been developed. For cell-based therapies, an important step is to develop defined and reproducible conditions for culturing hESCs. Thus, we are currently using the cellular microarray platform to identify optimum conditions for proliferation and expansion of hESCs.

Combinations of ECMPs (fibronectin, laminin, collagen I, collagen III, collagen IV, and collagen V), GFs (FGF, BMP-4, Wnt 3a, Wnt 5a), and glycans (heparin sulfate) were monitored for their effect on hESC growth and survival. Using real time microscopy and hESC lines designed to express fluorescent proteins we are able to monitor growth and survival over a period of 5 days. Multivariate analysis showed that specific microenvironmental components (collagen I, collagen IV, fibronectin, laminin, FGF, and Wnt 3a) displayed positive effects on hESC growth and survival. Meanwhile, other components (collagen III and collagen V) resulted in negative effects on hESC proliferation. Additionally, we identified several microenvironments that resulted in significantly increased hESC increased growth rates when compared to commercially available substrates.

In the future we will elucidate not only the culture conditions that will lead to the maintenance of hESCs but also the conditions required to promote the efficient differentiation of hESCs down a specific lineage.

SESSION 8: BIOREACTORS AND BIOPROCESSES FOR CELL EXPANSION AND DIFFERENTIATION

Invited Presentation

Novel Universal Cell-Based Drug from Adult Hematopoietic Stem Cells

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Adult hematopoietic stem cells have the ability to differentiate into all myeloid and lymphoid cell types. Using various cytokine combinations, these hematopoietic stem cells can be specifically differentiated ex vivo into different lineages. Cellerant has developed a novel technology to differentiate hematopoietic stem cells into myeloid progenitors that have applications, as demonstrated in animal models, in the treatment of neutropenia, enabling long-term hematopoietic engraftment with sub-optimal stem cell dose, and provide hematopoietic support in the event of accidental exposure to nuclear radiation. This off-the-shelf universal product, CLT-008, is capable of producing mature myeloid cells such as granulocytes, macrophages, platelets and erythrocytes *in vivo*. Furthermore, it has also been shown that these cells do not persist long-term and their function is independent of MHC restriction. A robust and reproducible production process has been developed that optimizes generation of myeloid progenitors from different donors and ensures a poled product of uniform composition. Scale-up of the process has been completed to initiate Phase 1 clinical studies. The key elements of process and pre-clinical development will be discussed in this presentation.

Oral Presentation

Manufacturing Stem Cells: Lessons Learned from 25 Years of CHO Cell Culture for Recombinant Protein Production

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Twenty five years ago, the first human clinical trial of a therapeutic protein made in recombinant CHO cell culture was initiated by Genentech. At the time, many viewed recombinant cell culture as a production method of last resort, certainly not one suitable to make a high-dose therapeutic. With low peak cell densities and low specific productivities in medium containing expensive serum and other animal-derived components, final product concentrations at harvest were relatively low. There were also safety concerns regarding oncogenes and retrovirus.

Twenty five years later, recombinant CHO cell culture has emerged as one of the dominant methods for production of recombinant proteins, especially high-dose therapeutic antibodies. Annual sales of products made by CHO cells now exceed $30 billion. To meet this huge demand, product concentrations at harvest have doubled every three years.

In this talk, the key challenges and technical achievements behind the success of CHO cell culture will be reviewed. For each key challenge met, the parallel challenge for stem cell culture will be presented. The relative difficulty of meeting the challenge for stem cells will be discussed. Adaptation of the approaches and technologies primarily developed for CHO cells will help us meet the challenges for stem cells. The potential long term impact on manufacturing costs will be discussed.

Oral Presentation

Large-Scale Production of Human Neural Precursor Cells in Computer-Controlled Suspension Bioreactors and Applications to the Treatment of Neuropathic Pain

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Transplantation of human neural precursor cells (hNPCs) represents a potential new treatment alternative for individuals suffering from incurable neurological disorders such as spinal cord injury. However, in order for cell restorative therapy to have widespread therapeutic significance, it will be necessary to generate clinical quantities of hNPCs under standardardized conditions. We report here the development of a serum-free growth medium (PPRF-h2) and scale-up protocols which allow for the generation of clinical quantities of human neural precursor cells in 500 mL computer-controlled bioreactors.

First, a cell growth medium previously developed in our laboratory for the expansion of murine neural precursor cells was successfully modified to support the expansion of hNPCs in culture (PPRF-h2 medium). The effects of supplementation with dehydroepiandrosterone (DHEA), basic fibroblast growth factor (bFGF), and human leukemia inhibitor factor (hLIF) on proliferation activity and neurogenic differentiation capacity were investigated using forebrain-derived hNPCs. Cells grown in the presence of DHEA retained their nestin expression, exhibited superior overall cell-fold expansion ($2.2x10^4$ versus $6.0x10^3$ over 70 days in culture), and differentiated into a greater proportion of neurons (44±2% versus 34±3%) compared to cells expanded in DHEA-free growth medium. Moreover, exposure to bFGF (20 µg/L) resulted in a high overall cell fold expansion ($9.6x10^6$ over 70 days in culture), and the generation of a large proportion of neurons (65±9%) following differentiation. This was superior to overall cell-fold expansion ($0.9±0.3x10^6$) and neuro-

genesis (41.3 ±3 %) observed in 10 µg/L bFGF but not significantly higher than overall cell-fold expansion ($7.0\pm4x10^6$) and neurogenesis (52.8 ±6 %) observed in 40 µg/L bFGF. Serial subculturing of the cells in 10 µg/L hLIF resulted in $6.7\pm1x10^{13}$ cell fold expansion, which was significantly higher than the overall cell fold expansion observed in the absence of hLIF ($2.1\pm0.9x10^{12}$) or 5 µg/L hLIF ($2.6\pm1x10^{13}$) but not higher than 20 µg/L hLIF ($7.7\pm3x10^{13}$). Using the modified cell growth medium, hNPC expansion was scaled-up from stationary culture to standard 125 mL suspension bioreactors. Surface aeration was used to meet the oxygen demand of the cells in the suspension bioreactors with a volumetric mass transfer coefficient (kLa) above 2.0 h-1. Furthermore, by controlling the shear field, the average hNPC aggregate diameter was maintained at target values of less than 600 µm, while maintaining the cell viability above 90%. The highest cell concentration of $3.35x10^6$ cells/mL was achieved at a moderate agitation shear of 100 revolutions per minute (rpm), during which the cells exhibited a doubling time of 85 h.
In order to demonstrate the feasibility of scale-up to large-scale suspension bioreactors, hNPC expansion was initially scaled-up to 250 mL suspension bioreactors using PPRF-h2 medium. In the 250 mL bioreactors, the cells exhibited a doubling time of 69 hours, reached a multiplication ratio of 44 after 16 days, and maintained a viability of approximately 90% over that same time period. We then engineered the protocols to further scale-up hNPC production to 500 mL computer-controlled suspension bioreactors. Standard biochemical engineering process control techniques were used in 500 mL bioreactor to control the temperature, pH and dissolved oxygen concentration at optimum levels of 37ºC, 7.3, and 70% air saturation, respectively. The cells exhibited a doubling time of 84 h, underwent a 36-fold expansion over the course of 18 days, and maintained an average viability of over 90%. Bioreactor-derived hNPCs retained their nestin expression following expansion and were able to differentiate into glial and neuronal phenotypes under defined conditions. Moreover, bioreactor-expanded hNPCs could be differentiated into a stable population of GABAergic cells.

We have recently shown that the hNPCs differentiated to a GABAergic phenotype prior to transplantation are able to restore functional behaviour in a rat model of neuropathic pain (allodynia)[1]. Allodynia develops following injury to the spinal cord or peripheral nerves due to the loss of inhibitory tone involved in spinal sensory function. To create the animal model, allodynia of the left hind paw was induced by unilateral L5-6 spinal nerve root ligation in rats and mechanical sensitivity was assessed. Transplantation of GABAergic cells into the spinal cord of allodynic animals resulted in a significant increase in paw withdrawal thresholds 1 week post-transplantation, and this behaviour was sustained for at least 6 weeks. Intraspinally transplanted predifferentiated GABAergic cells demonstrated *in vivo* immunoreactivity for the neuronal marker (β-III tubulin) and GABAergic marker (GABA). No indication of tumor formation was observed following transplantation of the bioreactor-expanded cells.

The results of these studies demonstrate that clinical quantities of hNPCs can be successfully and reproducibly generated under standardized conditions in computer-controlled suspension bioreactors and that these cells may have clinical application in the treatment of chronic pain syndromes.

References:

1. Mukhida, K.; I. Mendez, M. McLeod, N. Kobayashi, C. Haughn, B. Milne, B. Baghbaderani, A. Sen, L.A. Behie, and M. Hong, *Spinal GABAergic Transplants Attenuate Mechanical Allodynia in a Rat Model of Neuropathic Pain*, *Stem Cells*, **25**:2874-2885 (2007).

Oral Presentation

A Platform for Predictive Bioprocessing of Autologous Cells for Therapy Based on Automated Microwell Cultures

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The preparation of autologous cells for therapy requires the aseptic expansion and differentiation of stem cells at relatively small scale. For the maximum number of patients to benefit, methods for the parallel processing of large numbers of patient biopsies are required. This presentation outlines an automated microwell approach to cell expansion and modeling tools which will give clinicians an early indication of when sufficient cells will be available for implanting back into the patient.

The approach will be illustrated for the expansion of human Mesenchymal Stromal Cells (hMSC). These have the potential to differentiate into lineages of mesoderm origin, such as osteogenic, chondrogenic, and adipogenic lineages, presenting a promising potential for regenerative medicine applications. One of the major challenges associated with delivering hMSC to the clinic is the ability to propagate the cells in sufficient numbers for regenerative medicine therapies. Some of the reasons for this are, the low number of cells isolated from primary tissues, low growth rates *in vitro*, and the low population doubling limit of these cells before undirected differentiation and senesce occurs. The experimental studies describe the optimization of hMSC culture conditions in microwell formats and the use of a contained laboratory automation platform to help reduce the time required between biopsy and treatment. Results from related studies on the automated expansion and differentiation of mouse embryonic stem cells will also be shown for comparison.

In the case of hMSCs, cell growth kinetics were studied for cells isolated from frozen bone marrow samples from different donors and over sequential passages. Growth rates were found to be an intrinsic characteristic of the donor, decreasing consistently with increasing passage number, or population doublings. The overall duration of the cell expansion process in the automated platform was optimized by studying the effect of controlled parameters on cell growth kinetics and differentiation. The effect of inoculation cell density, feeding strategy, pH, and temperature on cell growth was determined, and optimum parameters were chosen to reduce the overall processing time needed to achieve the required number of cells for autologuous cell therapy. The quality of the final cell population was shown to be maintained throughout the cell expansion process based on cell surface marker expression.

For clinical applications the inability to predict the growth rate of isolated cells from a patient at each stage of the cell expansion provides a major obstacle towards the design of a time based automated bioprocess. In order to define processing times for the overall cell expansion of hMSC in an undifferentiated state, a simple mathematical model was also developed to describe the kinetics of growth for each passage based on the parameters obtained from passage one after hMSC isolation from an individual patient. This model can forward predict processing times at each passage for each donor, taking into consideration the decrease in growth rates associated with the increase in cell doublings. The validity of the model was tested with hMSC isolated from five different donors, and proven to be accurate in all cases.

SESSION 9: BIOMATERIAL SCAFFOLDS FOR STEM CELL THERAPEUTIC APPLICATIONS

Invited Presentation

Adult and Embryonic Stem Cells in Musculoskeletal Tissue Repair

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Stem cells, both adult and embryonic, have infused great excitement into the field of regenerative medicine and tissue engineering. However, challenges remain in how these potentially powerful building blocks can be leveraged to generate new tissues. We have applied both of these cell types to engineering musculoskeletal tissues after defining conditions to generate robust skeletal tissues from embryonic cells. Mesenchymal-like progenitor cells were generated from embryonic stem cells and cultured in biomaterials of varying composition. Cells encapsulated in hydrogels required incorporation of an adhesion peptide after which homogenous differentiation was observed. For osteogenic differentiation, the presence of mineral in a solid scaffold determined the differentiation pathway, via endochondral or intramembranous ossification mechanisms. Translation to relevant *in vivo* environments in the joint or cranium further confirmed the ability of the embryonic-derived cells to generate functional tissues similar to those of the native state. With judicious choice of delivery/biomaterial vehicle, these cells can be applied to a number of regenerative medicine applications.

Oral Presentation

Nanofibrous Scaffolds and Mesenchymal Stem Cells for Vascular Regeneration

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Synthetic nanofibrous scaffolds mimic the structure of native matrix fibrils, and provide guidance and microenvironment for cell infiltration and organization. Mesenchymal stem cell (MSC) is a potential source for vascular regeneration. Here we constructed small-diameter tissue-engineered vascular grafts (TEVGs) by seeding bone marrow mesenchymal stem cells (MSCs) into biodegradable nanofibrous scaffolds. The results from artery bypass experiments in rats showed that nanofibrous scaffolds allowed efficient infiltration of vascular cells and the remodeling of collagen and elastin matrix. Acellular grafts resulted in significant intimal thickening, while MSC-seeded grafts had excellent long-term patency and exhibited well-organized layers of endothelial cells and smooth muscle cells (SMCs) as in native arteries. Short-term experiments showed that nanofibrous scaffolds alone induced platelet adhesion and thrombus formation, which was suppressed by MSC seeding. In addition, MSCs, as ECs, resisted platelet adhesion *in vitro*, which was dependent on cell surface heparan sulfate proteoglycans. These results demonstrate the novel anti-thrombogenic property of MSCs and the potential of combining stem cells and nanomaterials for vascular tissue engineering.

To understand the effects of vascular mechanical factors on MSCs, we investigated how cyclic mechanical strain modulated the genetic reprogramming in MSCs. To simulate the vascular cell organization, MSCs were cultured on elastic substrate patterned with microgrooves and subjected to uniaxial mechanical strain in the direction of cell alignment. DNA microarray revealed global gene expression changes including an increase in the smooth muscle marker calponin 1, decreases in cartilage matrix markers and alterations in cell signaling. These effects were diminished if mechanical strain was applied in perpendicular to cell alignment. These results suggest that MSCs can sense the anisotropic mechanical stimulation and that vascular mechanical factors play an important role in MSC differentiation and function, which has important implications in vascular tissue engineering.

Oral Presentation

Tissue-to-Cellular Deformation Coupling in Cell-Microintegrated Elastomeric Scaffolds

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Introduction: Tissue engineering (TE) aims to recapitulate native tissue structure, composition, and mechanical function in a controlled and reproducible manner to overcome the limitations of traditional medical therapies. Moreover, the successful development of tissue engineered therapies rests on our ability to employ new materials, manufacturing and processing techniques, and control cellular mechanobiology. These are all predicated on a strong fundamental knowledge of native cellular and tissue function. This poses substantial difficulties since there are multiple length scales with complex architectures, modes of deformation, and biochemical stimuli which work synergistically to perform physiologic functions. It is generally accepted that both chemical and mechanical factors modulate cell biosynthesis when producing extracellular matrix. However, contemporary knowledge is lacking as to how externally applied traction forces are transmitted to the cell. Cells perceive and react to their mechanical environment is through direct cellular deformation. The complex cell-matrix interaction likely plays a critical role in the mechanotransduction of proteins for cell viability and proliferation and has direct implications towards the development of engineered tissues. Part of the reason for the continued limitation of our knowledge in this area is technical difficulties with performing such studies on native tissues, which can be confounded by complex hierarchical structures, vasculature, and multiple cell types.

We aim to address the relation between cellular deformation and deformations that occur at the organ or tissue level in a synthetic matrix densely integrated with cells. In the present study, a synthetic elastomer, poly (ester urethane) urea (PEUU), was chosen as the candidate scaffold material due to its biodegradable and cytocompatible properties With our unique ability to incorporate cells distributed throughout the scaffold via concurrent electrospraying of viable cells and electrospinning of PEUU fiber scaffolds, we are provided a unique platform to investigate the deformation response of cells seeded in a controlled fiber architecture *in situ*. In the present study, bone marrow derived stem cells (BMSC) were used. We hypothesize that cellular deformation within ES-PEUU scaffolds is likely a complex function of scaffold mechanical properties, architecture, and cellular coupling with the surrounding polymer fibers, all of which will influence BMSC response and ECM productivity. Our objective is therefore to quantify cell deformations in response to tissue-level scaffold strain, using cell nuclear aspect ratio (NAR), defined as the nuclei major axis over the minor axis, as an index of overall cellular deformation.

Results: Electrospinning produces continuous fiber scaffolds exhibiting a wide range of mechanical properties while providing a suitable surface for cell proliferation and growth. The electrospinning process employed in the current study manufactures a planar scaffold of sub-micron diameter non-woven PEUU fibers approximately 300-

400 nano meters thick to approximate the scale and mechanical behavior of native extracellular matrix while being able to undergo large deformations and fully recover. While electrospinning can fabricate scaffolds that possess an ECM structure, this morphology also results in pore sizes that are generally smaller (<5 microns) and more tortuous than those produced by other scaffold fabrication methods such as salt leaching and thermally induced phase separation. While it may be possible that cells statically seeded on electrospun matrices can migrate into the interior by displacing or enzymatically degrading individual fibers in the process, an extended culture period and appropriate signals for cell migration into thick construct interiors might also be required. So while electrospinning permits fabrication of biodegradable elastomers into matrices that can resemble the scale, architecture and mechanical behavior of the native ECM, achieving high cellular density and infiltration remains challenging and time consuming. To overcome this limitation, we utilized a technique to electrospray cells concurrently while electrospinning a biodegradable, elastomeric polymer, PEUU in a manner that allows the prescription of a degree of fiber alignment and scaffold anisotropy. This technique represents a reproducible and relatively rapid method to produce elastomeric fiber reinforced cellularized scaffolds which can mimic the biomechanical properties of native tissues while providing an environment conducive to cell viability. The use of NAR as a measure of cell deformation has been previously presented (see discussion). As validation for the use of this measurement technique for this application, TEM imaging was employed to quantify and compare nuclear and cell membrane dimensions of BMSC in electrospun (ES) PEUU scaffolds. Sections obtained from both the preferred fiber (PD) and cross-preferred (XD) fiber directions were measured separately. The major and minor axis lengths of the cell membrane and nucleus were quantified manually. NAR and cell membrane aspect ratios of cells in unstrained PEUU scaffolds were not observed to be significantly different in the PD or XD directions (pPD = 0.745 and pXD = 0.213).

The approach used in the current study centered on our ability to simultaneously quantify the cellular deformation (i.e. NAR) of living cells and changes in scaffold fiber architecture under biaxial deformation *in situ*. To first quantify the 3D geometry of the cell nuclei, image stacks were obtained using a laser scanning confocal microscope for 3 specimens in a free floating (or non-deformed) configuration and in states of increasing strip biaxial strain (¥åPD = 12, 50, 80 %) which is a special loading regime wherein the strain along one axis is increased while the orthogonal axis is constrained such that no deformation occurs. Strip biaxial strain was chosen as the primary mode of deformation as it induced large changes in NAR while preventing macroscale undulations from forming in the specimen thus allowing effective microscopic imaging. Additionally, biaxial modes of deformation better approximate physiological deformations of planar anisotropic tissues. From image stacks it was possible to ascertain general nuclei geometry and to quantify nuclei orientation in the specimen coordinate directions. The specimen coordinate frame is defined as the 3 orthogonal axes corresponding to the specimen's PD, XD, and the transmural direction through the thickness of the specimen. Cell orientation was quantified by determining the angle from the three principle nuclei directions to the specimen coordinate axes. Upon visual inspection, the cells appear to be primarily oriented in a planar configuration. Principle component analysis (PCA) confirms this observation wherein the imaged cells exhibit a scalene ellipsoid geometry (i.e. a>b>c) with the plane of the major and intermediate axis corresponding to the plane of fiber deposition. Similarly, when the vector defining the cell minor axis is summed for all measured cells (n = 20) in the reference configuration, the cells were observed to slightly digress from the specimen transmural direction by 5.92 ± 1.57.
As the cell-scaffold constructs underwent strip biaxial deformation, it was observed that the cells major principle direction rotated in the direction of deformation. The 3D cell orientation analysis also showed that the largest nuclear deformations occur in the PD-XD plane with little changes observed in the transmural direction. This is not a particularly surprising finding since the first two cell nuclear principle directions tended to be reasonable well aligned with the specimen axis (PD and XD respectively). However, since the nuclear deformations and kinematic changes occur in the construct plane, it is reasonable to simplify imaging and analysis modes from 3D to 2D without a measurable loss of important information. In fact, the results of our detailed 2D NAR results were in close agreement with the NAR-strain relationship seen in the 3D analysis. The simplified 2D mode of imaging also had the added benefit of reducing the time required for image acquisition which translates to more efficient use of each specimen whereby multiple states of deformation could be obtained.

Custom image analysis software was employed to quantify PEUU fiber orientation. Although the fabrication conditions were chosen to produce scaffolds with random fiber architecture, the specimens exhibited a slight preferred fiber direction in the unstrained state. In the non-deformed configuration, fibers were observed to be highly tortuous. Fiber tortuosity is defined as the ratio of the total fiber length to the fiber end-to-end length. For example, a perfectly straight fiber would have a tortuosity of 1 while fiber with many curves along its length will have a value greater than 1. The tortuous fiber morphology was also observed and quantified in scanning electron microscope (SEM) images of ES-PEUU scaffolds that did not contain BMSCs. As expected, a measurable increase in fiber alignment was observed with increased strip biaxial stretch. For specimens deformed in the XD direction, the fiber orientation distribution was observed to become more random with increasing strip biaxial deformation. Interestingly, an upper bound of fiber alignment was observed in specimens at high strain levels. Due to the interconnected architecture of the scaffold, the measured fiber orientation at high strains could not be described by an affine transformation. This upper bound correlated to a transition from an ensemble of tortuous fibers seen in the non-deformed specimen to a web-like architecture as manifested by a scaffold with straight, interconnected fibers.

Oral Presentation

Engineered Three-Dimensional Collagen-Based Extracellular Matrices Provide Microenvironmental Guidance of Endothelial Colony Forming Cell Proliferation Potential and Vascular Network Formation *in Vitro*

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Development of a functional vascular network is not only important for treatment of diabetes, peripheral vascular disease, and cardiovascular disease, it is also a major problem limiting current tissue engineering strategies targeting repair and regeneration of damaged or diseased tissues. Recently, endothelial progenitor cells (EPCs) have been identified as a cell population that is naturally released into the circulation and participates in physiological and pathological vessel formation *in vivo*. More recently, a specific subpopulation of EPCs,

termed endothelial colony forming cells (ECFCs), has been identified (Ingram et al., 2004). This subpopulation demonstrates a high proliferative potential as well as the ability to form functional vascular networks when implanted *in vivo*. For EPC-based therapies to reach their full clinical potential, transplantation/delivery systems that effectively and efficiently guide formation of functional vascular networks *in vitro* and *in vivo* must be defined and optimized.

This work targets the suspension of ECFCs in a liquid-phase, injectable, three-dimensional (3D) collagen extracellular matrix (ECM) microenvironment designed to self-assemble (polymerize) *in situ*. The matrix serves to both localize cells and provide them with local mechanical and structural cues that promote their survival and predictably modulate their fate, e.g. vascular network formation. The present study determined 1) the ability of two different type I collagen sources, pig skin collagen and a commercial grade collagen, to self assemble and form engineered 3D ECMs with a broad range of defined fibril microstructures and mechanical properties and 2) how specific engineered 3D ECM design parameters (e.g., storage modulus, fibril density) and initial cell seeding density affected the proliferation potential, differentiation, and 3D vascular network formation by ECFCs *in vitro*. Collagen source characterization showed that the polymerization kinetics, viscoelastic behavior, and fibril microstructure-mechanical properties of self-assembled matrices were distinct for the two different collagen sources. Interestingly, engineered ECMs prepared with pig skin collagen showed a substantially broader range of microstructure-mechanical properties compared to those prepared with the commercial collagen source. ECFCs, isolated from human umbilical cord blood, were then cultured within 3D collagen ECMs that varied in fibril density and storage modulus for periods of time up to 2 weeks. Analysis of ECFC response showed that variation of the 3D ECM microstructure-mechanical properties modulated vascular network formation, in terms of volume, distribution, and complexity of vascular structures, as well as shifted the population distribution of ECFC proliferative potential (measured using a single cell colony forming assay). Furthermore, vascular network formation could be achieved at a lower initial ECFC seeding density within engineered ECMs prepared with pig skin collagen.

These results demonstrate that tuning the mechanical and microstructural properties of an engineered 3D collagen ECM can influence resident ECFC behavior. This work provides valuable knowledge regarding critical design parameters for engineering instructive 3D ECM microenvironments that is not only applicable to ECFCs but also other stem/progenitor cells. Further translation of these findings for ECFC delivery *in vivo* offers potential benefit to patients with diabetic ulcers and critical limb ischemia, two complications that result in over 80,000 lower limb amputations annually, as well as cerebral ischemia and coronary artery disease.

Reference:

1. Ingram, D.A., Mead, L.E., Tanaka, H., Meade, V., Fenoglio, A., Mortell, K., Pollok, K., Ferkowicz, M.J., Gilley, D., and Yoder, M.C. (2004). Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. Blood **104**:2752-60.

Oral Presentation

Fibril Microstructure-Mechanical Design Features of Engineered 3D Collagen-Based Extracellular Matrices Modulate Mesenchymal Stem Cell Proliferation and Lineage Specific Differentiation

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Therapeutic use of stem and progenitor cells for the treatment of disease and dysfunctional tissue has been limited by the ability to control their survival, proliferation, and differentiation. Rational design of biomaterials and delivery systems for stem and progenitor cell therapies should be inspired by cell-instructive signaling modalities inherent to the *in vivo* stem cell microenvironment, or niche. Recently, the three-dimensional (3D) extracellular matrix (ECM) has been identified as a critical component of the stem cell niche that assists in guiding cell behavior. However, at present, it is not known what physical cues cells experience as they interact with the surrounding collagen fibrils and viscous interstitial fluid of the ECM. Lack of such knowledge is an important problem because it precludes the recapitulation of the 3D stem cell niche and the rational design of engineered biomaterials that include specific instructive signals to predictably and accurately direct stem cell behavior for improved tissue repair and replacement. Our laboratory has sought to determine the signaling modalities that occur locally at the 3D cell-ECM interface and how they influence cell fate. To achieve this goal we have utilized acid-solubilized type I collagen and defined polymerization (self-assembly) conditions that are quality controlled to yield highly reproducible 3D collagen ECM microenvironments with a broad range of tunable assembly kinetics (e.g. polymerization half-time), molecular compositions, and fibril microstructure-mechanical properties[1,3]. The advantages of these 3D collagen ECMs include physiologic relevance, inherent biological signaling capacity, precision-controlled design, and versatility in application. Previous work by our group[2] documented that fibroblasts adapt to the microstructure-mechanical properties of a 3D collagen ECM. They do so, in part, by altering their shape and size, organization of cytoskeletal components, distribution and size of cell-matrix adhesions, contractile force generated on surrounding collagen fibrils, and proliferation. In the present study, we expand this work to determine the ability of mesenchymal stem cells (MSCs) to differentially sense and respond to 3D collagen ECMs that varied in fibril density (fibril volume fraction) and mechanical properties. MSCs were entrapped at relatively low seeding densities within 3D collagen ECMs characterized with a range of fibril densities of 2% to 16%, storage modulus values of 13.44 Pa to 694.05 Pa, loss modulus values of 2.77 Pa to 59.09 Pa, and compressive modulus values of 3151.29 Pa to 15,828.73 Pa. Resultant 3D tissue constructs were cultured in "regular" or "adipogenic" medium for up to 2 weeks. Cell shape, organization of the cytoskeletal elements (microtubules and microfilaments), proliferation, and lineage specific differentiation were documented. Histochemical staining for lipid and expression of lipoprotein lipase (LPL) were used as indicators of adipogenesis, while calcium deposition and expression of core binding factor alpha1 (CBFA1) were used as indicators of osteogenic events. Results showed that variation of microstructure-mechanical properties of component collagen fibrils within a 3D collagen ECM modulated MSC shape and cytoskeletal organization. In addition, such alteration was sufficient to direct distinct growth and lineage-specific differentiation patterns of resident MSCs. It is especially noteworthy that such signaling via the local 3D collagen fibril microstructure and mechanical properties occurred for MSCs cultured in "regular" medium and did not require a specialized cocktail of soluble factors. Specifically, MSCs seeded within ECMs with a fibril density of 6% and storage modulus of 44.64±8.03 Pa readily proliferated and developed a mixed cell population including adipocytes and presumably undifferentiated, spindle-shaped cells. In contrast, MSCs seeded within ECMs with a fibril density of 16% and a storage modulus of 694.05±53.09 Pa proliferated less and developed a different combination of cell types

including minimal to no adipocytes, a decreased number of spindle-shaped cells, and focal aggregates of osteoblasts. Real time RT-PCR data for LPL and CBFA1 corroborated morphology and histochemical staining results. Culturing of the constructs in "adipogenic" medium exaggerated these ECM-dependent results. In fact, a 9-fold increase in the number of adipocytes was observed within constructs after 14 days of culture within low fibril density/low storage modulus ECMs in the presence of "adipogenic" medium. In contrast, MSCs cultured in high fibril density/low storage modulus ECMs in the presence of "adipogenic" medium showed only a moderate increase in adipogenic differentiation (approximately 2 times) but an 8-fold increase in the number of calcified bone nodules. Follow-up studies were conducted to determine if the initial seeding density affected the proliferative and lineage specific differentiation potential of MSCs within 3D engineered ECMs. For these studies, MSCs were seeded in high fibril density/high storage modulus ECMs at densities ranging from $0.5x10^4$ cells/ml to $50x10^4$ cells/ml and the constructs were cultured in either "regular" or "adipogenic" media. In general, decreasing the cell seeding density caused a decrease in cell-cell interactions, an increase in cell-ECM interactions, a decrease in adipogenesis, and an increase in osteogenesis, independent of culture medium. In fact, when seeded at the lowest cell density, MSCs grew as focal regions and expressed an osteogenic phenotype, with little to no evidence of other cell types. As the initial seeding density increased, a cell population of mixed phenotypes developed. At the highest cell density, adipocytes and undifferentiated MSCs were prominent with no evidence of osteogenesis. In summary, the significance of this work expands the understanding of stem cell biology within 3D culture formats. In addition, these results assist in defining design criteria for the development of "instructive", self-assembled, collagen-based 3D ECMs (generally referred to as 3D engineered ECMs) that can predictably control cell behavior and contribute to the development of functional tissues and organs for both research and clinical applications.

References:

1. Brightman, A.O., Rajwa, B.P., Sturgis, J.E., McCallister, M.E., Robinson, J.P., and Voytik-Harbin, S.L., Time-lapse confocal reflection microscopy of collagen fibrillogenesis and extracellular matrix assembly *in vitro*, *Biopolymers*, **54**, 222-234 (2000).

2. Pizzo, A.M., Kokini, K., Vaughn, L.C., Waisner, B.Z., and Voytik-Harbin, S.L., Extracellular matrix (ECM) microstructural composition regulates local cell-ECM biomechanics and fundamental fibroblast behavior: a multidimensional perspective, *J. Appl. Physiol*., **98**:1909-1921 (2005).

3. Roeder, B.A., Kokini, K., Sturgis, J.E., Robinson, J.P., and Voytik-Harbin, S.L., Tensile mechanical properties of three-dimensional type I collagen extracellular matrices with varied microstructure, *J, Biomech, Eng*., **124**:214-222 (2002).

3-D Hydrogel Microenvironments for Liver Progenitor Cells

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The development of cell-based therapies for the liver is hindered by the limited proliferation and instability of function exhibited by hepatocytes *in vitro*. Promising alternative cell sources include liver progenitor cells, which demonstrate both bipotential properties and the capacity for expansion in culture. We have explored the behavior of a model progenitor cell type, bipotential mouse embryonic liver (BMEL) cells for applications in tissue engineering. It is known that the differentiation of these cells is sensitive to so-called '3D cues'; therefore, we have examined the role of biochemical signals presented in 3D contexts of both synthetic and natural hydrogel microenvironments. Use of photopolymerizable PEG hydrogels as the synthetic platform also enabled generation of arrays of microgels for parallel analysis. Using these tunable platforms, we have explored the role of cell-cell interactions, soluble growth factors and presentation of extracellular matrix in a 3D context, specifically identifying the role of cell-cell interactions in differentiation along the hepatocyte lineage. Further examination of liver progenitor responses within versatile hydrogel systems will continue to provide insights into fundamental aspects of liver progenitor biology and liver development.

A Novel Treatment of TEL/AML1 Induced Acute Lymphoblastic Leukemia Using siRNA Expressing Autologous Stem Cell Transplantation in Patients

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Acute Lymphoblastic Leukemia (ALL), a cancer of the White Blood Cells, is a hematological malignancy wherein the malignant clone tends to crowd out the normal cellular components of the bone marrow. If left untreated, it is a lethal disease that can rapidly affect other vital organs[1]. It accounts for approximately 73% of leukemia cases in children in the age group of 1-15yrs. The TEL/AML1 chimeric gene, also known as ETV6/CBFA2, is the most common gene rearrangement and chemotherapy is the standard treatment[2].

Vinca alkaloids have now been in use for over 30 years, the anthracyclines and L-asparaginase for 25 years, and the epipodophyllotoxins for almost 20 years. New purine analogues have proved effective against chronic leukaemias in adult patients, but appear much less promising for childhood ALL[3]. Further therapies also include radiation therapy and bone marrow transplants. Histone De-Acetylase (HDAC) inhibitors like suberoylanilide hydroxamic acid, trichostatin A and butyric acid, can effectively inhibit the cancer but despite a high success rate in the above treatments, relapses are common[4]. Approximately 20% of the patients with relapsed B-cell ALL expressed the TEL-AML1 gene transcript[5]. It is in response to this need for more effective treatments that we intend to use the advances in stem cells and siRNA technology to silence the TEL-AML1 fusion transcript, which has been shown to induce apoptosis of the cancerous cell.

siRNA's are short RNA duplexes which when introduced into a cell inhibit the translation of the target mRNA. The antisense strand forms an RNA Induced Silencing Complex (RISC) and cleaves the mRNA such that it cannot be translated. The sense strand, also known as the passenger strand, is degraded[6]. Thus by using suitably designed siRNA's to knockdown the TEL-AML1 fusion transcript which have demonstrated significant ability to also induce pro-apoptotic pathways, growth of leukemic cells can be effectively inhibited.

The appropriate siRNA sequences have been successfully designed and tested by Diakos *et. al.*[2], and based on their ability to influence gene expression, a functional sequence and a control sequence was chosen. Within 6 days of treatment with the functional siRNA, all fusion protein was depleted, and after another 2 days 60% of the cells underwent apoptosis as compared to only 20% of the cells treated with control siRNA. The pro-apoptotic signals were initiated through a mechanism involving heat shock proteins (HSP) 70 and 90[2].

Thus, the real challenge pertains to delivering the engineered siRNA to its site of action. Although the use of lipid and polymer based gene delivery systems have shown promise in recent studies, they are unable to over-come the cellular barrier. They are also not robust enough to reach the nuclear membrane, and the timely release of the gene cannot be controlled[7]. Adenoviral and retroviral vectors have also been considered as possible vectors, except they are still in controversy over their transfection efficiency[7]. Stem cells, on the other hand, have been gaining much approval.

Stem cell Research, after the approval of Proposition 71, is finally being accepted in society, albeit gradually[8]. Studies have shown that stem cell transplantation, performed as support for high-dose chemotherapy; improves both the response rate and survival in myeloma over that obtained with conventional chemotherapy[9].

The Center for International Blood and Marrow Transplant Research (CIBMTR) estimates that approximately 4,700 stem cell transplants of various types were performed in patients with myeloma in North America in 200310. The advantage of using allogenic stem cells is that there is little or no immunogenic reactions, which would be the case if exogenic stem cells were to be used10. Another advantage would be that the siRNA will be produced *in vivo* and thus eliminating the need to periodically administer it into the patient.

This paper will review the current therapies for ALL, their drawbacks and the consequent need for new therapies of which the therapeutic use of stem cells is most promising. The advantages, disadvantages and major obstacles to implementing this mode of treatment will also be discussed at length. Finally, in this paper, we will examine the feasibility of using allogenic haematopoietic stem cells transfected with siRNA directed to silence TEL/AML1-induced ALL, and induce apoptosis in cancer cells with this aberration. This mode of therapy could be used for adults and children.

References:

1. Hoelzer, D. et al., Acute Lymphoblastic Leukemia., Hematology Am. Soc., Hematol. Educ. Program., 162-92 (2002).
2. Diakos, C. et al.; RNAi-mediated Silencing of TEL/AML1 Reveals a Heat Shock Protein and Survivin Dependent Mechanism for Survival, *Blood,* **109**:2607-2610 (2007).
3. Newell, D., How to develop a successful cancer drug – molecules to medicines or targets to treatments, *European Journal of Cancer*, **41**:676-682 (2003).
4. Butler, M.B. et al, Suberoylanilide Hydroxamic Acid, An Inhibitor of Histone Deacetylase, Suppresses the Growth of Prostate Cancer Cells *in Vitro* and *in Vivo*, *Cancer Research*, **60**:5165-5170 (2000).
5. Endo, C, Oda M, Nishiuchi R, Seino Y., Persistence of TEL-AML1 transcript in acute lymphoblastic leukemia in long-term remission, Pediatrics International **45**:275–280 (2003).

6. Venturini L., M. Eder, M. Scherr, RNA-Mediated Gene Silencing in Hematopoietic Cells, *Journal of Biomedicine and Biotechnology*, 1-13 (2006).
7. Mahato, I.R. et al, Cationic Lipid and Polymer-based Gene Delivery to Human Pancreatic Islets. **7**:89-100 (2003).
http://www.smartvoter.org/2004/11/02/ca/state/prop/71/ Accessed on 11th Nov. 2007.
8. Avivi, I., J.M. Rowe and A.H. Goldstone, Stem Cell Transplantation in Adult ALL Patients, *Best Practice & Research Clinical Haematology*, **15**:653-674 (2003).
9.http://www.multiplemyeloma.org/treatments/3.03.php Accessed on 11th Nov. 2007.

AC Electrokinetic Microstirring for Enhancing Stem Cell Growth Cultures

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AC-driven electrothermal flow (ETF) is proposed to significantly enhance the throughput of stem cell growth cultures. Large-scale culturing of human embryonic stem cells (hESCs) and inducing controlled differentiation is a labor-intensive and time-consuming process. The long periods in between media changes and lengthy time for entire process of cell differentiation may be due to diffusion limitations of the nutrients to the cells on the bottom surface of the dish. AC electrokinetic forces are used to generate electrothermal flow, which in turn produces a circular stirring fluid motion that enhances the transport of diffusion-limited particles. Thereby, augmenting diffusive transport of nutrients using AC electrothermal flow may provide useful for decreasing necessary incubation time and increasing throughput in stem cell cultures.

Previous work has demonstrated experimentally and numerically that AC electrothermal microstirring can enhance temporal performance of the heterogeneous biotin-streptavidin assay by nearly an order of magnitude. ETF is the result of a conductivity gradient, produced by localized Joule heating, which interacts with an applied electric field. ETF, which acts on the bulk fluid and dominates in higher conductivity fluids, is amenable for microstirring biological fluids (i.e. the culture-rich media). Such a high conductivity culture media poses a concern that the local temperature rise may adversely affect the hESCs. However, preliminary cell viability tests were performed on Hs27 cells and it is concluded qualitatively that heat from electrothermal microstirring does not adversely affect the cells. These results show promise for the potential use of electrothermal microstirring to enhance nutrient.

An AFM Approach for Studying Human Embryonic Stem Cell Differentiation

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Human embryonic stem (hES) cells offer immense therapeutic potential. Although hES cells have unlimited self-renewal power, they tend to differentiate spontaneously in culture. Identification of the initial stage of differentiation is essential in maintaining well-defined population of undifferentiated stem cells and in directing differentiation along specific cell lineages. We have applied the atomic force microscopy (AFM) in characterizing protein-protein interactions in a single cell on the nanometer scale to capture the initial stage of hES cell differentiation. For instance, we found that the distribution of TrA-1-81 antigen is heterogeneous with a population of ~8500 epitopes per undifferentiated cell, and closely associated pairs of epitopes were frequently observed; however on an initially differentiated cell, the distribution of TrA-1-81 is homogeneous and random with a population of < 500 epitopes per cell. We also probed the mechanical properties of hES cells. Our preliminary data show that undifferentiated cells are softer than undifferentiated cells, and are more dependent on cell-cell "contact". Such research will facilitate the overall understanding of hES cell biology and will assist the strategy development for effective prevention and correction of unwanted differentiations in hES cell self-renewal and for effective differentiation.

Baculovirus as a New Gene Delivery Vector for Stem Cell Engineering and Bone Tissue Engineering

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Baculovirus has emerged as a promising gene delivery vector. Herein we aimed at exploring the potentials of baculovirus in genetically engineering mesenchymal stem cell (MSC) for ectopic *in vivo* bone formation. We first determined the transduction conditions of MSCs for sustained, high level production of active bone morphogenetic protein 2 (BMP2) using a recombinant baculovirus (Bac-CB) and evaluated the osteogenesis. Our data indicated that the MSCs could be efficiently transduced by Bac-CB at a multiplicity of infection (MOI) of 40 and produced BMP2 at a level up to 9 ng/ml. The BMP2 expression could be prolonged via supertransduction, which accelerated osteogenesis of MSC *in vitro* as determined by ALP assay and alizarin red staining. The transduced MSCs were encapsulated in alginate gel *in vivo* via subcutaneous injection into the back of nude mice to evaluate whether the baculovirus-transduced MSCs enabled ectopic bone formation. At 2 weeks postinjection, the majority of injected MSCs differentiated into osteocytes and exhibited little bone formation. At 6-week postinjection, abundant, highly mineralized bone was formed *in vivo*. These data, for the first time, demonstrate that the potent osteogenesis and *in vivo* bone formation from baculovirus-transduced MSCs, and implicate the potentials of baculovirus for safe gene therapy and bone tissue engineering.

BMP2, FGF2, and Dlx3 in Tissue-Specific Dental Stem Cell Differentiation

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Stem cell differentiation into target dental tissues is characterized by complex tissue-specific changes in gene profiles facilitated by factors such as BMP2, FGF2, and Dlx3. Objectives: To characterize trends in tissue-specific cell differentiation toward dental follicle (DF), dental pulp (DP), and periodontal ligament (PDL) lineages as they relate to BMP2 and FGF2 growth factors and the Dlx3 transcription factor. Methods: Cells were treated with either BMP2 (80ng/ml), FGF2 (10ng/ml), or a combination of both for a period of 6 days in order to test the effect on cultured MSC, DF, DP, and PDL progenitor cells. To determine the effect of Dlx3 knockdown, MSC and PDL were transfected with Dlx3 siRNA. After 18 days culture, total RNA was extracted and expression of collagen I, Dlx3 and osteocalcin was evaluated by real time RT-PCR. To quantify mineralization, cell cultures were stained with Alizarin Red S (AR-S). Results: FGF2 and BMP2 significantly upregulated collagen I in MSC (4.5-fold for BMP2, 2-fold for

FGF2, and 4.7-fold for BMP2 and FGF2 together) while downregulating collagen I in DP (1.37, 1.1, and 3.14-fold, respectively) and PDL (2.39, 1.64, and 3.48-fold, respectively). Microarray comparison indicated significantly higher Dlx3 expression in DF, DP and PDL compared to MSC. Dlx3 knockdown caused a significant reduction in mineralization parameters: AR-S staining was reduced by 27-56% depending on cell type and osteocalcin gene expression by 3.15-7.12-fold. Conclusion: These data indicate that BMP2 and FGF2 upregulated collagen I gene expression in MSC cells and display the opposite effect in DP and PDL cells, suggesting that the effect of growth factors on matrix gene expression depends on the progenitor cell differentiation status. Dlx3 knockdown significantly reduced mineralization indicators, suggesting a role for Dlx3 in the mineralization and differentiation of dental tissues. Support by NIDCR grant DE15425 to TGHD is gratefully acknowledged.

Characterization and *in Vivo* Endocrine Differentiation of Mesenchymal Islet-Derived Precursor Cells

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A shortage of donors for islet transplantation for the treatment of diabetes has led to intensive research into new cell sources. A promising line of research is the isolation, expansion and directed endocrine differentiation of progenitor cells from the adult human pancreas. Human islet-derived precursor cells (hIPCs) are proliferative cells that grow out from human islets in the presence of serum and can be expanded for about 20 population doublings. hIPCs are mesenchymal stem cells (MSCs), since they adhere to plastic, express MSC markers (CD75/CD90/CD105) and differentiate *in vitro* into mesodermal lineages such as adipocytes, chondrocytes and osteocytes. Moreover, hIPCs have activated β-catenin signaling as demonstrated by the presence of nuclear β-catenin, high expression of several β-catenin target genes and decreased proliferation upon knockdown of β-catenin. Expanded hIPCs do not express insulin mRNA and form epithelial cell clusters following serum withdrawal. When ECCs were transplanted to NOD/SCID mice, most of the ECC preparations matured into endocrine (insulin and glucagon-producing) cells that secreted C-peptide in response to glucose. Thus, hIPCs are a pancreas-specific type of MSC that could be potentially used for cell replacement therapy in Type I diabetes.

Controlling Early Human Embryonic Cell Fate through Manipulation of Exogenous and Endogenous Signaling

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in vivo, early germ layer development and primitive streak formation in the human embryo is controlled by transforming growth factor-â (TGFâ) and Wnt signaling, but further tissue patterning requires precise spatial control of these signaling pathways through secreted inhibitors. *in vitro*, it has been shown that exogenously added TGFâ and Wnt ligands are able to induce primitive streak-like development from human embryonic stem cells (hESC); however, these cytokines also trigger the secretion of agonists and inhibitors to these same signaling pathways. Importantly, how these added cytokines and endogenously secreted inhibitors interact to control hESC differentiation has yet to be elucidated. Here we demonstrate that, in a serum-free environment, two days of treatment using both Activin A and bone morphogenic protein-2 (BMP-2) efficiently promotes primitive streak-like gene and protein expression from hESC in a dose-dependent manner. Following 2 days of treatment using 50 ng/mL of Activin A and BMP-2 we find that 25.6 ± 0.9 % of cells stain positively for T-Brachyury, a transcription factor known to be expressed in the primitive streak, by immunohistochemistry. This was further confirmed through gene expression using quantitative reverse transcription-polymerase chain reaction where we measured a 58 ± 17 and 44 ± 13 fold increase in transcript levels of the primitive streak markers T-Bry and Mixl1, respectively, in samples treated with both Activin A and BMP-2. It was also found that treatment with either Activin A or BMP-2 induces the expression of Wnt and TGFâ signaling ligands such as Wnt3 and Nodal as well as their inhibitors, namely LeftyB, Cerberus, and Dickkopf1. Moreover, by controlling the size of differentiating hESC colonies using microcontact printing we can modulate the expression level of these endogenous signaling agonists and antagonists. It is hypothesized that this effect is mediated through endogenous paracrine signaling whereby increased endogenous paracrine signaling has a greater effect in larger colonies due to higher local cell density when compared to smaller colonies. Considering that primitive streak cells have the potential to form both mesoderm and endoderm lineages, we believe that modulating endogenous protein expression through colony size will consequently control the fate of the bipotential primitive streak-like cells. These results demonstrate that the differentiation of hESC towards mesoderm and endoderm can be tightly controlled through the addition of defined growth factors while concurrently controlling colony size.

Development of a Defined Medium for the Expansion of Human Mesenchymal Stem Cells for Use in Clinical Applications

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The utilization of human mesenchymal stem cells (hMSCs) for therapeutic tissue regeneration has attracted a great deal of attention over the last decade because they (i) can be safely isolated from easily accessible sources such as bone marrow, (ii) can be readily expanded in culture, and (iii) are multipotent and have the capacity to give rise to cell types found in many different tissues. Conventional media used for isolating and expanding hMSCs are typically supplemented with fetal bovine serum (FBS). While clinical studies involving hMSCs expanded in FBS containing media have already started, the use of FBS represents a major obstacle for the clinical implementation of hMSC related therapies as it is poorly defined (varies in quality and composition), may contain prion, viral, or zoonotic agents, and can elicit immune reactions. The use of human serum has been investigated as an alternative to FBS. Whereas autogenic serum has been shown to be able to support expansion, it can not be obtained in quantities sufficient to generate clinical numbers of hMSCs. Moreover, allogenic human serum has been shown to cause hMSC growth arrest and death. Thus, we initiated a study aimed at developing a new chemically-defined, serum-free medium which could be used to isolate and expand hMSCs for clinical applications. Important growth factors and protein components were tested, and resulted in the development a novel defined medium (PPRF-msc medium) that could support long-term culture of hMSCs derived from bone marrow. Cells isolated and expanded in the defined PPRF-msc medium were compared with those obtained using a widely used FBS-containing medium (i.e., DMEM

supplemented with 10% prescreened FBS) with respect to colony-forming units, cumulative populations doublings, adipogenic and osteogenic differentiation potential, and immunophenotype. We found that PPRF-msc medium supported a 7.29 x 10^9 cell-fold expansion within 30 days in culture (plating density of 150,000 bone marrow mononuclear cells per cm2 for primary culture and 5,000 cells per cm2 per passage). This was significantly higher than the 1.17 x 10^6 cell-fold expansion achieved over the same time period using the FBS-containing medium. PPRF-msc medium also resulted in an increase in colony-forming unit fibroblast (CFU-F) frequency as well as CFU-F size. Cells expanded in this medium retained their adipogenic and osteogenic differentiation potential. Specifically, a calcium deposition assay for the differentiated cultures showed that cells expanded in either PPRF-msc medium or FBS-containing medium retained comparable osteogenic potential. Similarly, the AdipoRed assay indicated that cells grown in those media retained a comparable adipogenic differentiation capacity. Finally, cells isolated and expanded using PPRF-msc medium expressed high levels (>99%) of CD13, CD29, CD44, CD73, CD90, CD105, CD166 and HLA-ABC and were negative for CD14, CD19, CD34, CD45, and HLA-DR. We have also shown that PPRF-msc medium can also support the isolation and expansion of hMSC-like cells from the human pancreas. Our defined culture condition should provide a robust platform that will ease the clinical implementation of hMSC based therapies.

Development of Artificial Hematopoietic Stem Cell Niche *in-Vitro* Using Biomaterial Microarrays

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Throughout the lifespan of a tissue and organ, stem cells are maintained in their 'niches', which are interactive microenvironments designed to facilitate cellular differentiation in a spatiotemporal manner. Stem cell niches are composed of extracellular matrix molecules (ECM), cell signals and other soluble factors that facilitate stem cell differentiation. For instance, hematopoietic stem and progenitor cells (HSPCs), reside in specific niches that control survival, proliferation, self-renewal, and differentiation in the bone marrow. Specific HSPC niches enable continuous trafficking of the cells between the bone marrow and blood compartments for the maintenance of normal hematopoiesis. Using a novel Microplate Biomaterial Microarray (MBMTM) technology, we have created an artificial hematopoietic stem cell niche *in vitro* with characteristics ideal for cultivation and directed differentiation of human embryonic stem cells into progenitor cells of hematopoietic lineage. The niche allows combinatorial uploading of cells, genes, growth factors, extracellular matrices, antibodies, and small molecules to support, promote, enhance and direct growth and controlled differentiation of stem cells. Furthermore, using "smart" modern biomaterials, niche components are uploaded in a kinetic fashion (i.e. slow, fast, and inducible). The presentation will provide an overview of the technology. In addition, most recent findings for conditions effective in the following paradigms will be presented: a) Hematopoietic differentiation of human ES cells, b) Development of a rapid drug screening assay for cancer stem cells, c) Ex-vivo enhancement of human bone marrow cells (BMC) for treatment of Myocardial Infarction, d) Growth, propagation, and differentiation of human cardiac stem cells for cardiovascular regenerative therapy, e) Small molecule induced de-differentiation of human fibroblasts into iPS cells.

Directed Differentiation Using Applied Physical Forces

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Embryonic stem cells, like all cells, are influenced by their microenvironment, including both chemical and physical cues. *in vitro*, these cues can serve to influence stem cell fate (e.g., maintain stem cells undifferentiated or promote differentiation along a pathway) and/or to facilitate regenerative medicine applications (e.g., expand stem cells to large numbers or promote uniformly differentiated populations). In this project, the focus has been on controlling the cellular microenvironment of mouse embryonic stem cells (mESCs) by applying physical forces.

Physical forces, such as compression, tension, and shear, have long been applied to cells via bioreactors. Many bioreactors used with stem cells are motion-based suspension cultures meant to accelerate and augment cell expansion kinetics and capabilities. These vessels often apply a spatially-dependent complex profile of shear stress. In order to better design scale up bioreactors for either stem cell expansion or differentiation, there is a need to use well-defined physical cues to better understand the effects of fluid shear stress on cellular processes. Studies in our lab have shown that when fluid shear stress was applied to mESCs, the overall expression of Flk1 increased and the cells formed structures on matrigel, outcomes characteristic of endothelial cells. By using similar model systems to apply well-defined mechanical cues, we better understand the influence of the physical microenvironment on embryonic stem cells differentiation.

Effects of Membrane Cholesterol on Elongation of Human Mesenchymal Stem Cells Grown in Topographically Patterned Surfaces

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Mesenchymal stem cells (MSCs) have received special attention in stem cell engineering because of their multi potency. Thus, many efforts have been focused on how to control the growth and differentiation of MSCs. Recently, it was unveiled that chemical factors as well as physical factors, such as topography or stiffness of substrates influence on their growth, morphologies and differentiation. However, the mechanism explaining these changes has been not clearly presented. In this study, we present that membrane cholesterol concentration greatly influence on the extent of elongation of cells grown on topographically patterned surfaces. For example, cells whose membrane cholesterol was depleted with 2.5mM Methyl- beta-cyclodextrin elongated three times more than cholesterol enriched cells on a 1 micron groove patterned surface. These results suggest that cholesterol level of MSCs is an important factor for decision of their morphology in differentiating lineages.

Evaluation of Differentiation Methods Using Embryonic Stem Cells with Endothelial Specific Markers

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Endothelial cells are being investigated for many tissue engineering applications including small diameter vascular grafts and pre-vascularized tissue beds. However mature endothelial cells are limited in passage number. Additionally there is new evidence that endothelial cells from one tissue may not be appropriate for endothelialization of another.

For this purpose we have evaluated the use of embryonic stem cells (ESCs) to provide high quantities of endothelial precursors in various stages of differentiation. The endothelial cells in this study were selected using genetically transformed mouse ESCs with a GFP/Puror fusion protein under the control of different endothelial promoters (Flk-1, PECAM, tie-1, and VE-Cadherin). Cells were differentiated under multiple conditions including 2-D culture, 3-D embryoid bodies, and continuous shear environments. The use of a range of promoters enabled puromycin selection of cells at various stages in endothelial differentiation; Flk-1 at day 5 (D5), PECAM at D6, tie-1 at D7, and VE-Cadherin at D8. After 7 days of additional selection in puromycin, the surviving cells were characterized for endothelial phenotype by Dil-Ac-LDL uptake and immunostaining of PECAM and vWF. Additionally the relative levels of RNA expression following differentiation were evaluated by RT-PCR, evaluating for expression of Flk-1, PECAM, vWF, VE-Cadherin, tie-1 and GFP. This study demonstrated that these genetically modified ESCs can be used to provide a high yield of endothelial cells and precursors under varying differentiation conditions. Also these cells show marker expression comparable to control cells including bovine aortic endothelial and human umbilical vein endothelial cells.

Expansion and Neural Commitment of Mouse Embryonic Stem Cells on a Microarray Platform

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Embryonic stem (ES) cells hold great promise as renewal source of cells for regenerative medicine and tissue engineering. However, the factors that regulate cellular events, such as expansion and differentiation, are not completely known. Microscale technologies enable the development of platforms that can be used in tissue engineering applications and high-throughput screening, aiding in the task of elucidating the signals that affect ES cells. A particularly promising application is the development of cell-based microarrays for the growth and differentiation of stem cells, and for investigating the influence of small molecules and cell growth conditions on cell physiology and function.

In our work, we have developed a microarray platform that is suitable for stem cell expansion and differentiation, and that can be used for the rapid and efficient tracking of stem cell fate and quantification of specific stem cell markers. The microarray system consists of murine ES (mES) cells encapsulated in alginate gel spots with volumes as low as 20 nL. A non-contact microarrayer was used to spot the cells on poly(styrene-co-maleic anhydride) functionalized glass slides. The spotted cells were expanded in serum-free medium in the presence of leukaemia inhibitory factor (LIF) and bone morphogenic protein-4 (BMP-4), showing high levels of the pluripotency marker Oct-4 after 5 days. Furthermore, kinetic parameters such as specific growth rate or doubling time were calculated and found to be comparable with values obtained for culture plates and spinner flasks. We also examined the neural commitment of mES cells on the microarray. We observed the generation of neuroectodermal precursor cells, which were characterized by expression of the early neuronal marker Sox-1, whose levels were measured *in situ* using a green fluorescent protein reporter system. The developed microarray system is a versatile tool for ES cell studies and high-throughput screening, with potential applications in drug discovery assays.

Fabricating 3-D Hydrogel Scaffolds Using Stereolithography for Stem-Cell Differentiation

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The principle method of tissue engineering is to grow cells *in vitro* in a suitable scaffold. Cells alone cannot grow in any defined orientation without the proper mechanical and biochemical cues provided by a scaffold. It is well known that stem cells express higher differentiation efficiencies when cultured on 3D scaffolds. There are many conventional scaffold fabrication techniques in practice, including textile technologies, solvent casting, particulate leaching, membrane lamination, and melt molding. However, it remains extremely difficult to control the 3D shape of these scaffolds. We have explored the potential of using stereolithography (SL) for tissue engineering applications by fabricating complex scaffolds out of photopolymerizable poly(ethylene glycol) hydrogels. SL is a liquid rapid prototyping system that uses light to fabricate objects layer-by-layer from a computer-aided design image. It makes fabricating higher-ordered structures with internal architecture possible without the difficulties of conventional scaffold fabrication techniques. Encapsulating stem cells into these structures will allow further insight into the role of 3D cues in stem-cell differentiation and tissue engineering.

Functional Characterization of Contractile Properties of Human Embryonic Stem Cell-Derived Cardiomyocytes

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Several studies have investigated gene and protein expression in human embryonic stem cell (hESC)-derived cardiomyocytes. However, effective cardiac therapies using stem cells require the cells to develop functional contractile properties, which may depend on the protein and cytoskeletal structure as well as its expression. In order to provide a measure of cardiomyocytes functionality, we have developed a method employing dynamic traction force microscopy for quantitatively measuring the contractile force produced by hESC-derived cardiomyocytes. In this method, cells are attached to a soft polyacrylamide substrate that contains fluorescent beads. As the cell beats and deforms the substrate, the beads are tracked and their displacement is transformed into a map of stresses on the substrate surface, developed by the cell. This method was tested using the NIH hESC line H9 which had been previously modified with an insertion of a puromycin resistance gene regulated by the cardiac αMHC promoter and a blasticidin resistance gene regulated by the Rex promoter, expressed in undifferentiated pluripotent hESCs. These cells were allowed to differentiate for 10 days before addition of puromycin to select for cardiomyocyte precursor cells expressing αMHC. At 13 days after selection, contractile "cardiospheres" were dissociated with trypsin and plated at low density on bead-containing polyacrylamide gel coated with type I collagen and with an elastic modulus of approximately 10 kPa. This method allows comparison of the magnitude, time course and spatial

distribution of cell contraction. Preliminary findings show that some cells contracted with over 1μN total axial force, in the range of control cardiomyocytes isolated from neonatal rats. The general shape of the contraction curve appears very similar to those of neonatal rat ventricular myocytes with similar time constants.

Gaussia Luciferase -- A Novel Bioluminescent Reporter for Tracking Stem Cells Survival, Proliferation and Differentiation in Vivo

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Transduction of bone-marrow derived human mesenchymal stem cells with lentivirus vectors expressing a novel and naturally secreted bioluminescent reporter was undertaken as an approach to track stem cells survival, proliferation as well as differentiation using bioluminescent imaging techniques. A self inactivating lentivirus vector expressing both Gaussia luciferase (a novel secreted luciferase that is over 2000 times brighter than firefly or Renilla luciferase) and green fluorescent protein (Lenti-Gluc-GFP) was used for these studies. Transduction of human mesenchymal stem cells with this lentivirus vector at an MOI of 30 resulted in approximately 100% transduction as assessed by GFP fluorescence. Luciferase activity in the conditioned media was found to be directly proportional to the number of stem cells suggesting that Gluc could be a useful marker for assessing stem cells growth and survival *in vivo*. Stem cells-expressing Gluc mixed with matrigel and implanted subcutaneously in nude mice could be easily visualized and tracked over time using standard *in vivo* bioluminescence imaging. Since Gaussia luciferase is naturally secreted, the extent of cell survival and proliferation *in vivo* could be assessed by measuring its levels in few microliters of blood. Similarly, circulating stem cells (injected intravenously) could also be monitored over time by measuring the activity of Gluc in the blood.

We have also used a Gaussia luciferase based reporter plasmid systems (pSiscreen system from Targeting Systems) for the effectiveness of siRNAs in silencing target genes in stem cells. Once an siRNA "hit" is found, it can be co-transfected to stem cells expressing Gluc and the effect of gene silencing on stem cell survival or fate *in vivo* can be monitored by *in vivo* bioluminescence imaging together with quantitative assessment of Gluc activity in the blood. We will present our results on successful tracking of small numbers of injected/implanted stem cells using *in vivo* bioluminescence imaging as well as ex-vivo Gluc-blood measurements. We will also present some preliminary results on the utility of this system for studying cell differentiation.

High Efficiency Generation of Epithelial Progenitors from Human Embryonic Stem Cells under Defined Conditions

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Stratified epithelia continually regenerate throughout life to maintain tissue function and homeostasis. Epithelial cells play important roles in wound healing and secretion and are frequently involved in cancer. While transgenic animal models have provided substantial insight into epithelial development, reliable human epithelial cell systems are needed. Given their enhanced proliferative capacity and pluripotency, human embryonic stem cells (hESCs) are an attractive source of normal epithelial cells. However, current methods of differentiating hESCs to epithelial lineages such as skin keratinocytes are inefficient and require feeder cells and/or undefined medium components. Here we employ quantitative analysis of differentiated hESC populations to identify key signaling factors involved in ectodermal lineage specification, demonstrating a novel, stage-specific effect of retinoic acid (RA) on hESC differentiation. RA mediates expression of the epithelial transcription factor p63 and, in conjunction with bone morphogenetic (BMP) signaling, efficiently directs hESCs to epithelial rather than neural fates. This differentiation process can be effectively used to generate essentially pure populations of non-transformed epithelia under feeder-free, defined conditions. Furthermore, hESC-derived keratinocytes are capable of expressing terminal differentiation markers of the skin and cornea when presented with the appropriate cues. This method may therefore be used to produce functional epithelial progenitors for diagnostic and therapeutic applications.

High-Throughput Screening of Gene Function in Stem Cells Using Clonal Microarrays

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The study of individual gene function and the interconnectivity between different genes is critical to understand the mechanisms that regulate stem cell behavior. We have developed a high throughput microarray-based approach for gene function screens in stem cells using clonal microarrays. The method can be used for both loss and gain of function screens, and is compatible with both adult and embryonic stem cells. Within the space of a standard microscope slide, clonal microarrays allow us to assay the effect of approximately 3500 different genetic sequences on various stem cell functions, including signaling, differentiation, and proliferation. After screening, select clonal cell populations can be isolated directly from the microarrays and expanded for further analysis. To demonstrate the utility of this approach, we have used clonal microarrays to screen for genes that enhance adult neural stem cell proliferation. Clonal microarrays could facilitate large-scale gene function screening in a variety of mammalian cell types, as well as expedite the identification of novel drug targets and the generation of novel stem cell therapies.

Human Mesenchymal Stem Cell Construct Development in a 3-D Perfusion Bioreactor System: Effects of Interstitial Flow and Morphogen Distribution

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Adult mesenchymal stem cells are a powerful candidate cell type for tissue regeneration. We have developed a perfusion bioreactor system that provides a controlled culture environment, and is an important tool in stem cell expansion and tissue engineering. The process of creating functional 3-D tissues constructs in the bioreactor system using stem cells and scaffolds is influenced by the dynamic culture environment characterized by dynamic stresses and concentration gradients of essential nutrients and regulatory molecules. Among these microenvironmental parameters, dynamic stresses and pressure gradients are cru-

cial regulators of 3-D hMSC tissue development, which impart forces on the cells and affect cell behavior by transporting solutes and shaping the extracellular distribution of key signaling proteins. Our previous study has found that hMSCs actively respond to shear stresses at a range of $1x10^{-5}$ to $1x10^{-4}$ Pa, orders of magnitude lower than those observed on planar surfaces (Zhao et al., 2007). However, the effects of culture microenvironment in the perfusion bioreactor system on construct development require further investigation to determine the physiological microenvironment and its effects on hMSC development. In this study, we have combined the experimental and modeling approaches to delineate the effects of shear stress and distribution of morphogens. First, the effects of interstitial flow on the developmental characteristics of hMSCs grown in 3-D poly(ethylene terephthalate) (PET) matrices were determined by operating the modular perfusion bioreactor system developed in our lab in either transverse or parallel perfusion modes (Zhao and Ma, 2005). The two perfusion modes have different hydrodynamic characteristics but identical volumetric flowrate of 0.2 mL/min, residence time, and initial cell seeding population. A 1.6 times higher proliferation rate, higher CFU-F formation, and stem cell gene expression at day 20 were observed in parallel perfusion in comparison with those under transverse perfusion. The interstitial flow also upregulated the osteogenic differentiation potential at day 20 as measured by the osteonectin gene expression and calcium deposition in the matrices. In addition, ECM of hMSCs was patterned differently under the two perfusion modes, in which transverse perfusion directed a graded ECM distribution along the flow direction, as opposed to a denser and more uniform spatial patterning of ECM on both sides of 3-D constructs dictated by parallel perfusion flow mode. Hydrodynamic modeling results showed cells in transverse flow mode exposed to media flow at ~20 μm/s and shear stress in the range of $1x10^{-3}$ to $9x10^{-3}$ Pa. Concentration gradients of TGF-β in the construct under various flow conditions are being analyzed to determine the spatial distribution of morphogens on hMSC proliferation and differentiation. TGF-β superfamily members are known morphogens and have profound effects on hMSC proliferation and osteogenic differentiation. Combining modeling and experimental results will not only help to delineate the effects of the biomechanical microenvironment and morphogen transport but also improve tissue engineering strategy using perfusion bioreactor systems.

References:

1. Zhao, F., R. Chella and T. Ma, Effects of shear stress on 3-D human mesenchymal stem cell construct development in a versatile perfusion bioreactor system: experiments and hydrodynamic modeling, *I,* **96**:584-595 (2007).

2. Zhao, F. and T. Ma, *Biotechnology and Bioengineering*, **91**:482-493 (2005).

Human Mesenchymal Stem Cell Differentiation in Response to Matrix Stiffness and Transforming Growth Factor-β1 May Be Regulated by HDAC Activity

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Human mesenchymal stem cells (MSCs) isolated from the bone marrow have the ability to differentiate into the cells of many tissue types, such as bone, cartilage, fat, tendon and muscle. In order to use MSCs for stem cell therapies, we must better understand their differentiation pathways and the microenvironmental factors involved in regulating MSC differentiation. Among the important factors in a cell's microenvironment is matrix stiffness, found to regulate migration, proliferation and more recently, stem cell differentiation. In this study, we investigated the crosstalk between a chemical factor, transforming growth factor-β1 (TGF-β1), and a mechanical factor, matrix stiffness, in MSC differentiation. TGF-β1 is important in vascular development and the modulation of smooth muscle cell (SMC) phenotype. We have previously reported that TGF-β1 induced higher expression of smooth muscle (SM) markers in MSCs on polystyrene. In matrix stiffness studies, MSCs expressed less SM markers with decreasing matrix stiffness, while TGF-β1 did not induce SM markers in MSCs on softer substrates but did on stiff substrates. On the other hand, a soft matrix upregulated chondrogenic markers in MSCs, while augmenting the TGF-β1-induced increase of these markers compared to on a stiff matrix. This finding shows that the same growth factor can have two different effects on the same cell depending on the stiffness of the substrate. To study the involvement of various pathways in rigidity-dependent MSC differentiation, MSCs were treated with chemical inhibitors. Interestingly, HDAC inhibitor caused a decrease in alpha-actin and increase in collagen II gene expression, indicating that the expression of these rigidity-dependent markers could be regulated by HDAC. Next we studied the activity of HDAC in MSCs grown on different rigidities and observed that HDAC activity decreased with decreasing rigidity, indicating that a decrease in HDAC activity could be responsible for the decrease in SM markers and increase in chondrogenic markers on softer substrates . This study stresses the importance of matrix rigidity in stem cell differentiation and corresponding growth factor effects, as well as proposes the possible involvement of HDAC in regulating this phenomenon.

Human Mesenchymal Stem Cells Gene Expression of Osteogenic Markers in a 3D Environment

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Introduction: Human mesenchymal stem cells (hMSCs) are being intensively investigated in bone tissue engineering due to their high proliferative potential, their default osteogenic differentiation pathway, and their major role in endochondral and intramembranous bone formation *in vivo*. The use of hMSCs in tissue engineering strategies requires a biocompatible and nonimmunogenic vehicle, capable of providing for cell localization and fate. Alginates, natural polysaccharides extracted from seaweed, are linear copolymers of 1,4-linked α–L-guluronic and β–D-mannuronic acids, have been widely proposed as cell immobilization matrix since they can be tailored through relatively simple chemical modifications.

The present work aims to investigate the RNA expression level of hMSC immobilized in RGD-modified alginate for several osteogenic markers in the presence of osteogenic inductors: alkaline phosphatase (ALP), bone sialoprotein (BSP), collagen type I (COL 1), osteocalcin (OCN) and the transcription factor Cbfa-1.

Materials and Methods: hMSCs (Cambrex, USA) were cultured with D-MEM (Gibco) with 1%P/S e 10%FBS for basal conditions. For osteogenic conditions cells were cultured in the presence of the same medium supplemented with β–glycerophosphate, ascorbic acid and dexamethasone. Alginate was modified through grafting of RGD peptides to promote biofunctionality and further irradiated and oxidized to increase cell viability and promote biodegradability1. To immobilize hMSCs in alginate microspheres, a cell pellet was homogenized with the 2 wt-% RGD-modified alginate in 0.9 wt-% NaCl solution (20x106 cells/mL of alginate). Subsequently, the alginate-cell suspension was extruded under a coaxial nitrogen-flow using a Var J1 encapsulation unit (Nisco). Microspheres were allowed to form in an isotonic 0.1 M CaCl2 solution for 10 min and finally rinsed in TBS and in culture medium. Microspheres were maintained at 37ºC in dynamic culture conditions during 4 weeks.

At weekly time points a microsphere suspension was transferred to a 24 well-plate and cells were released by adding 50mM EDTA solution in PBS and total RNA was extracted using the RNeasy Mini Kit (Qiagen). For cDNA synthesis, 0.5 µg of total RNA was reverse-transcribed by SuperScript II Reverse Transcriptase (Invitrogen) and qRT-PCR experiments were then performed using an iQ5 (Bio-Rad). The housekeeping gene glyceraldehyde 3-phosphate dehydrogenase was used as the endogenous assay control. The expression value for each target gene was normalized to the day 1 in each group.

Results and Discussion: In basal conditions among the genes studied ALP, Cbfa1 and COL increased progressively along the course of the experiment. BSP and OCN decreased from the 1st to the 7th day and then increased until day 21. In osteogenic conditions the gene expression is less regular. ALP and COL increased until day 14th and then decreased. On the other hand, Cbfa1 and OCN have the opposite behaviour, decreasing until day 14th and then increasing. BSP gene has an irregular expression. Although gene expression behaviour is different for both conditions, overall, the gene expression values are higher for cells in the presence of osteogenic inductors. According to Aubin2 Cbfa1 is expressed during all the differentiation steps, although its expression can vary, increasing during preosteblast stage and reaching a high detectable level in differentiated osteoblast. ALP expression is detectable in mature osteoprogenitors, increasing until differentiated osteoblast. Col I starts to be detectable in mature osteoprogenitors, and maintains the same expression levels until differentiated osteoblast stage. BSP is transiently expressed during the osteoblast development and has a heterogeneous expression in individual cells. OCN appears with high expression levels latest on post-proliferative osteoblast.

Hence, by analysing the RNA expression obtained, the results suggest that cells are responding to osteogenic inductors and entering in the differentiated osteoblast stage. Overall, the present results suggest that RGD grafted alginate is capable to support osteogenic differentiation, although complementary work needs to be done (such as metabolic and ALP activity).

References:

1. Evangelista, M.B., S.X. Hsiong, R. Fernandes, P. Sampaio, H.J. Kong, C.C. Barrias, R. Salema, M.A. Barbosa, D.J. Mooney, P.L. Granja, *Biomaterials*, **28**:3644-55 (2007).
2. Aubin, J.E., *Endocr. Metab.Disord*, **2**:81-94 (2001).
3. Acknowledgements: Portuguese Foundation for Science and Technology (FCT) for PhD grant SFRH/BD/22307/2005 and project POCTI/SAU-BMA/55556/2004.

Hydrodynamic Mixing Conditions Imposed by Rotary Orbital Culture Modulate Embryonic Stem Cell Differentiation

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Embryonic stem cells (ESCs) have the unique potential to differentiate into all somatic cell types, however effective use of ESCs for regenerative cell-based therapies requires an improved understanding of environmental mechanisms regulating ESC differentiation. Currently, most directed differentiation schemes rely primarily on application of exogenous factors to stem cell cultures, but controlling multiple environmental factors, including mechanical as well as biochemical stimuli, may result in increased stem cell differentiation efficiency. The objective of this study was to examine how hydrodynamic mixing conditions imposed by rotary orbital shaking culture can be used to modulate ESC differentiation. Mouse embryoid bodies (EBs) cultures were initiated and maintained in suspension for 7 days at 25, 40, or 55 rpm continuously or at various combinations of the different rotary mixing speeds. Morphometric analysis of the resulting EBs was performed to assess the average size and variance of the population, and histological analysis, as well as real-time PCR studies, were performed to assess the effects of variable rotary speeds on ESC differentiation. Rotary speed was inversely proportional to EB size for EBs maintained continuously at one rotary speed. EBs from slower rotary speeds (25 rpm) exhibited a more frequent cystic appearance, while increasing rotary speed produced EBs with a denser cell nuclei appearance. In general, EBs formed at slower rotary speeds exhibited higher mesendoderm gene expression and enhanced cardomyogenesis compared to the faster rotary speeds. Varying rotary speed after initial EB formation at one speed was capable of further modulating EB size, morphology and gene expression patterns, indicating that EBs remain sensitive to hydrodynamic mixing effects during the course of suspension culture. Altogether, these results indicate that the hydrodynamic mixing environment created by rotary orbital culture is capable of differentially regulating ESC differentiation, suggesting a new potential design criteria that could be integrated into the creation of bioreactors for stem cell bioprocessing.

Hypoxia Prolongs the in Vitro Lifespan of Human Mesenchymal Stem Cells and Modulates Cell-Cell Interactions

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Low oxygen tension is an integral component of the human mesenchymal stem cells native bone marrow microenvironment. Changes in oxygen concentrations in the *in vitro* culture of stem and progenitor cells affect many of their innate characteristics. Recent studies have shown that proliferation, apoptosis and differentiation of stem cells are all affected at reduced oxygen concentrations. In this study, human mesenchymal stem cells (hMSC) were grown in two-dimensional (2-D) conventional tissue culture plates and in three-dimensional (3-D) polyester terephthalate (PET) constructs and were maintained under an atmosphere containing 2% oxygen as this is considered more akin to the *in vivo* microenvironment of these cells. In 2-D experiments, reduced oxygen concentrations resulted in approximately 30-fold higher hMSC expansion over 6 weeks. Importantly, under hypoxia, hMSC do not proliferate more quickly, but maintain their growth-rates for extended periods relative to normoxic samples, and do not exhibit typical contact inhibition characteristics resulting in the formation of multiple cell layers. At the latter stages of each passage, hypoxic hMSC displayed changes in the cell and nuclear morphologies as well as enhanced ECM formation and organization. These changes in cellular characteristics and interactions in the hypoxic cells were accompanied by considerably increased expression levels of connexin-43, a protein used in gap junction formation, indicating that cell-cell communication (and possibly signaling) is modified at reduced oxygen tensions. These changes in cellular characteristics were also accompanied by higher mRNA levels of Oct-4, Rex-1, and HIF-2α. Importantly, after 7 passages under the two oxygen conditions, the cells appear to maintain their multi-lineage differentiation capacity. When grown in 3-D scaffold, hMSC exhibited an extended

lag phase in order to acclimatize to culture conditions. However, they subsequently proliferated continuously throughout the culture period, while maintaining significantly higher colony-forming unit capabilities and expressing higher levels of stem cell genes than hMSC cultured at 20% O2 (normoxic) conditions. Upon induction, hypoxic hMSC also expressed higher levels of osteoblastic and adipocytic differentiation markers than normoxic controls. Hypoxia induced increased total protein levels in hMSC throughout the culture period, as well as significantly different fibronectin expression patterns suggesting that oxygen levels can significantly affect tissue-development patterns. The results from this study demonstrate that oxygen concentrations ubiquitously affect many aspects of stem-cell physiology, including growth and *in vitro* development and are an essential element of the *in vivo* hMSC niche. Oxygen environment is a critical parameter for directing hMSC fate and function and is to be considered in the development of engineered constructs.

References:

1. Grayson, W. L., F. Zhao, B. Bunnell, and T. Ma, Hypoxia enhances proliferation and tissue formation of human mesenchymal stem cells, *Biochemical and Biophysical Research Communications*, **358**:948–953 (2007).

2. Grayson, W. L., F. Zhao, R. Izadpanah, B. Bunnell, and T. Ma, Effects of Hypoxia on Human Mesenchymal Stem Cell Expansion and Plasticity in 3D Constructs, *Journal of Cellular Physiology*, **207**:331-339 (2006).

Imaging Differentiation-Induced Embryonic Stem Cells

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Our primary goal is to create an imaging platform to determine whether therapeutically administered stem cells differentiate into the desired phenotype *in vivo*. Currently, one of the clinical treatments under investigation for the repair of ischemic cardiac tissue is direct stem cell injection. Both hematopoietic and mesenchymal stem cells are being investigated, with the hope that hematopoietic stem cells (HSCs) differentiate to endothelial cells which lead to angiogenesis, while mesenchymal stem cells (MSCs) should lead to the formation of new cardiomyocytes. These treatments have shown varying levels of success; however, it is unclear whether this is due to cellular differentiation to the desired phenotype, delivery of a different cell type which is still capable of promoting a healing response (e.g. macrophages), or delivery of molecules present in the cell suspension (e.g. G-CSF). Consequently, we are examining the use of tissue-specific promoters to drive expression of marker genes *in vivo* thereby conclusively demonstrating differentiation to that phenotype.

In our experimental setup, we will examine the use of embryonic stem cells (ESCs) which we have evaluated for endothelial differentiation in previous studies. For this study, ESCs will be generated that express two complementing fragments of b-galactosidase; one, a constitutively expressed protein fragment and the other, a differentiation-specific protein fragment. When cells are undifferentiated, they express only the constitutive protein fragment and do not re-create a full length active enzymatic protein. When cells are induced to differentiate, both protein fragments will be expressed to produce a full length active b-galactosidase. Full-length b-galactosidase activity can then be visualized either by colorimetric assay or fluorescence. Plasmids were constructed which contain either a constitutively active promoter (b-actin) or an endothelial-specific promoter (tie-1). These promoters were used to drive the production of a single b-galactosidase subunit, termed either alpha-4 or omega, respectively. Plasmids were electroporated into mouse embryonic stem cells and stable clones were selected using a separate drug selection cassette (*neo* for the tie-1 constructs, *zeo* for the ?-actin constructs). Once stable clones are generated, they will be evaluated undifferentiated or differentiated following a 2-D endothelial differentiation protocol. In parallel, the constructs will be tested in cells which are known to be positive for tie-1 expression (bovine aortic endothelial cells) or negative for tie-1 (3T3 fibroblasts). Our data demonstrate that co-expression of these fragments will result in b-galactosidase complementation that can be observed by substrate hydrolysis of Xgal. This imaging platform is truly innovative and will ultimately enable *in vivo* functional imaging of ESC growth and differentiation.

Inducible Enzyme Replacement in the MPSII Brain via Microcapsule-Based Delivery of Genetically Engineered Neural Stem Cells

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Hunter syndrome (Mucopolysaccharidosis Type II, MPSII) is an X-linked lysosomal storage disorder, caused by an absent or deficient enzyme, Iduronate-2-Sulfatase (IDS). Hunter Syndrome is a lethal disorder, limiting patient life span in its most severe manifestation to between 10 and 20 years. Even with systemic recombinant enzyme replacement (Elaprase®, Shire), the brain continues to degenerate as IDS is excluded from passage across the blood-brain barrier. Towards realizing a complementary therapy for the brain, a polymeric microcapsule-based system serving the dual purpose of advanced *in vitro* culture system as well as vehicle for *in vivo* delivery of Neural Stem Cells (NSCs) has been developed. *in vitro*, murine NSCs expand rapidly (doubling time ≈ 20 hours) in homogeneous populations (Nestin+ fraction = 94.9 ± 4.1 %) within methylated collagen/alginate-based microcapsules. erative acute inflammatory response that is typically following surgical lesioning of the mouse brain (C57BL/6 IDS knock-out mouse, MPSII mouse). NSCs are genetically engineered to inducibly secrete the enzyme alginase via tetracycline-controlled transcriptional activation for cued microcapsule degradation, freeing encapsulated NSCs on demand *in vivo*. NSCs are similarly genetically engineered to inducibly secrete IDS via ecdysone-controlled transcriptional activation for enzyme replacement in the MPSII brain. NSC migration is tracked *in vivo* by imaging of fluorescently-labeled chitosan-iron oxide nanoparticle conjugates which are rapidly uptaken by cells in culture prior to implantation. These nanoparticle conjugates enable multimodal imaging (i.e. MRI, fluorescence microscopy) of implanted NSCs.

Neural Stem Cells are primitive precursors to the parenchymal cells

of the brain. They are able to self-renew, and also to differentiate into neurons, astrocytes, and oligodendrocytes, both *in vitro* and *in vivo* 1,2. However, two presently unresolved challenges hinder widespread use of neural stem cell-based therapies. The inability to achieve timely NSC expansion without loss of multipotency results in a scarcity of transplantable NSCs available for patients in need. And delivery of NSCs by implantation of protease-treated cells into tissue that is acutely inflamed post-operatively is very ineffective3,4.

NSC fate is determined by a variety of factors; however, the importance of microenvironmental cues has in previous studies proven paramount. Cell-instructive, methylated collagen-based polyelectrolyte microcapsules have been developed for efficient expansion of NSCs *in vitro*.homogeneous populations (Nestin+ fraction = 94.9 ± 4.1%, KI67+ fraction = 89.6 ± 3.7%), suited for generation of clinically relevant numbers of NSCs for therapeutic implantation. No significant differentiation is observed in microcapsule culture; however, on planar controls, cells express glial markers, indicating significant lineage specification during the same duration of culture. Such cell-instructive microcapsules may also serve to sequester NSCs during the post-operative inflammatory response.

The post-operative condition of the brain during the acute inflammatory response to surgical lesioning has been shown to elicit astrocytic differentiation of NSCs, resulting in their contributing to scar tissue formation4,5. Methylated collagen/alginate microcapsules are designed to protect and sequester NSCs within a microenvironment enhancing phenotype maintenance during the acute phase of this inflammatory response, saving the cells from exposure to the surrounding, inflamed tissue. After acute inflammation has passed, secreted alginase is to decompose the NSC-containing microcapsule wall, freeing NSCs to respond to recruitment signals from the surrounding brain tissue. For this purpose, NSCs were retrovirally transduced to inducibly express the ectopic, abalone-derived enzyme alginase. Secreted alginase and nuclear-localized DsRed-Express are expressed under tetracycline-control (pRevTet-On® vector, Clontech) in an inducer dose-dependent manner. NSCs are freed *in vivo* from microcapsules by non-invasive, per-oral administration of doxycycline.

Efficiency of double transduction, necessary to achieve tetracycline-inducible gene expression, was nearly 45%. The alginase gene construct was designed to include two 3' influenza virus hemagglutinin-A (HA) epitope tags for immune detection (e.g. ELISA) preceded by an Ig β-chain leader sequence promoting enzyme secretion from the cell. DsRed-Express is co-expressed from the same vector via a Polio virus internal ribosomal entry site (IRES), and is nuclear localized by importin-mediated transfer to the nucleus. ty of the system in NSCs is observed over a broad range of doxycycline concentrations, spanning hundreds of picograms per milliliter to micrograms per milliliter, in a dose-dependent manner. Furthermore, transductants remain tetracycline-responsive beyond differentiation (by withdrawal of growth factor supplementation and addition of 2.5% fetal bovine serum to NSC media). NSC-secreted alginase degrades alginate microcapsules within hours *in vitro*, demonstrated by release of immobilized, very high MW (2000 kDa) FITC-dextran from a degrading alginate microcapsule over time. Analogous to genetic engineering for inducible expression of alginase, NSCs are currently engineered to similarly express and secrete IDS under control of a second blood-brain barrier-permeable chemical inducer, RSL1.

Currently, all technologies are established and tested with mouse NSCs. Work in the near future will focus on adaptation of protocols and methods to manipulation of human NSCs.

References:

1. Reynolds, B.A. and S. Weiss, *Science*, **255**:5052 (1992)
2. Eriksson, P.S., et al., *Nat. Med.,* **4** :11 (1998)
3. Carpenter, M.K., et al., *Exp. Neurol.*, **158**:2 (1999)
4. Park, K.I., et al., *Nat. Biotech.,* **20**:11 (2002)
5. Molcanyi, M., et al., *J. Neurotrauma,* **24**:4 (2007)

Magnetic Resonance Microscopy for Monitoring Stem Cell Regeneration in Vitro

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Magnetic resonance microscopy (MRM) is used to monitor the regeneration of stem cells in tissue-engineered constructs. Measurements of the growing stem cells' MR relaxation times (T2 and T1rho) were conducted over a 4-week growth period using an 11.74 T Bruker system. Comparing with the T2 relaxation time, T1rho shows a significantly early decrease after only two weeks of the regeneration of stem cells. The measured MR parameters are correlated with histologically monitored stem cells. This study shows that T1rho measurement can provide quantitative data with which to characterize the regeneration of stem cells and it is superior to the conventional T2.

Molecular Mechanisms of Adult Neural Progenitor Proliferation and Self-Renewal

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Adult hippocampal neural progenitor cells (AHNPCs) can generate nearly all major cell types within the mammalian brain, including neurons, astrocytes, oligodendrocytes, and endothelial cells, which makes them promising candidates for the treatment of neurological injuries and diseases. Additionally, adult neurogenesis may play roles in learning and memory, stress and depression, response to injury, and aging. Because the processes of AHNPC proliferation and self-renewal appear to be significant regulatory points for adult neurogenesis, the purpose of this work is to determine 1) how extracellular mitogens activate intracellular signaling networks mediating AHNPC proliferation and 2) whether mechanisms that promote proliferation also promote self-renewal. We have found that the PI3K/Akt pathway, which is broadly important for self-renewal of multiple stem cell types, is vital in mitogen-induced AHNPC proliferation. Specifically, Akt stimulation is sufficient to induce cell proliferation and inhibit differentiation. However, PI3K stimulation is not sufficient to induce proliferation, implying that mechanisms other than PI3K are necessary for Akt activation. Furthermore, the transcription factor CREB is a downstream mediator of Akt signaling and promotes AHNPC proliferation. These results provide insight into the signaling network that regulates adult neural progenitor cell proliferation and self-renewal, eventually leading to stem-cell based therapies.

Mouse Embryonic Stem Cell Expansion in a Microcarrier-Based Stirred Culture System

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Ex vivo expansion of mouse embryonic stem (mES) cells in a microcarrier-based stirred culture system was performed aiming at the scaling-up of cell expansion in adherent conditions while maintaining their pluripotency and neural commitment potential. The 46C mES cell line was used as a model system. This cell line contains a green fluorescent protein (GFP) gene knocked at the locus of the early neuroectodermal marker gene Sox1, allowing the monitoring of neural commitment (GFP expression) by fluorescent microscopy and flow cytometry.

The scale-up of 46C mES cell expansion was studied in spinner flasks (30-50 mL), in serum-containing (DMEM/FBS) or serum-free (SF) medium, both supplemented with LIF. In order to immobilize mES cells, two different microcarrier-based culture systems under stirred conditions were evaluated: a solid (microporous) gelatin-covered microcarrier (Cytodex 3) and a porous gelatin microcarrier (Cultispher S). It was found that both microcarriers were a suitable matrix for the expansion of mES cells under stirred conditions. This could be observed either by direct microscopic observation of the cells immobilized onto the beads (after DAPI and MTT staining), or indirectly through the calculation of cell numbers achieved during culture in time. Firstly, the expansion of mES cells (5x104 cells/ml) on Cytodex 3 (0.5 mg/ml) was performed using DMEM/FBS medium in a spinner flask culture system, for 8 days, being half of the medium replaced every two days. The maximum cell concentration achieved was (1.9±0.1)x106 cells/ml on day 6, which represented a 38±2-fold increase. The maximum specific growth rate was 1.4 day-1, which corresponds to a doubling time of 0.5 day. In the next step, the expansion of mES cells was performed in the spinner flasks on the Cultispher S microcarriers. This microcarrier was used since its macroporous nature would potentially provide a more protective environment to the cells, favouring cell expansion. The cells were also inoculated at a initial cell density of 5x104 cells/ml on Cultispher S (1 mg/ml) both in the presence of DMEM/FBS and SF medium. The culture was performed during 8 days with half of the medium being replaced everyday. This feeding regimen was advantageous in comparison to the 50% medium replacement every two days used for the Cytodex 3 experiments, due to daily removal of spent medium. The maximum cell densities achieved during 8 days in culture were (2.6±0.7)x106 and 3.5x106 cells/ml, which corresponds to fold increases of 50±15 and 70 for DMEM/FBS and SF, respectively. For both media, the maximum specific growth rate was 1 day-1 which corresponds to a doubling time of 0.7 day. Concerning the doubling times, it was observed that td (Cytodex 3) was inferior to td (Cultispher S), which is consistent with the fact that the "start up" of the culture was faster for the cells expanded on Cytodex 3. This behaviour might be due to the fact that cells need more time to adhere and to start their proliferation on the Cultispher S. Indeed, first the cells will adhere to the outer surface of the macroporous microcarriers and only then will start to migrate into the interior of the gelatin matrix. However, the macroporous Cultispher S seemed to be advantageous in providing a more protective environment against shear stress forces, which harmful effects are exacerbated in serum-free conditions. Concerning the metabolic characterization of mES cells expansion on spinner flasks, waste accumulation seemed to be the likely cause for the slowing down of the cell growth at the end of the cultures since in the cultures where lactate concentration was above 20 mM or where ammonia was above 2 mM, cell proliferation was reduced. Indeed, these concentrations were found to be inhibitory in other mammalian cell cultures. In addition, it can be considered that the feeding regimen performed in all cultures circumvented nutrients limitations since glucose and glutamine concentrations were never less than 5 and 0.6 mM, respectively. During these experiments, a key role upon culture starting was found for glutamine since the maximum consumption of this aminoacid was observed after 1-2 days in culture. Of notice, after 8 days of culture under stirred conditions in SF medium, only 8% of the cells were apoptotic and less than 3% were necrotic. Therefore, approximately 90% of the expanded cells were viable. More importantly, the cells maintained the ability to differentiate into neural progenitors with more than 90% of Sox1-GFP+ cells obtained upon the neural commitment protocol. In addition, after expansion, cells stained positively for alkaline phosphatase, indicating that after 8 days in culture, a high percentage of cells remained pluripotent in their undifferentiated state.

Nanoparticles for Multimodal Tracking of Implanted Neural Stem Cells *in Vivo*

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Tracking living Neural Stem Cells (NSCs) *in vivo* after implantation enables real-time monitoring of NSC fate and migration. One approach is to load cells with imaging-contrast enhancing nanoparticles for MRI or CT imaging of cells. MRI tracking can be accomplished by introducing superparamagnetic iron oxide (SPIO) nanoparticles with superior relaxivity into cells. Cells with a sufficiently high intracellular density of these nanoparticles are recognizable as localized regions of hypointensity in T*2-weighted MRI images. With high field strength (e.g. >11 Tesla), small animal research-scale imaging tools, MRI can report in-plane cellular locations at nearly the single-cell scale (e.g. 40µm x 40µm)1.

An obstacle hindering application of such tracking technology to a wide variety of cell types is low uptake efficiencies of such particles *in vitro*. Towards enhancing uptake for superior imaging contrast, carboxylated SPIO nanoparticle surfaces (Ocean Nanotech, USA) were functionalized with chitosan, previously shown to promote cellular uptake of DNA for non-viral gene therapies through endocytosis2. Chitosan, deacetylated chitin from crustacean shells, is a polycationic, linear polysaccharide of ß-(1-4)-linked D-glucosamine (deacetylated) and N-acetyl-D-glucosamine. Chitosan spontaneously complexes with the negatively charged carboxylated nanoparticle surface, enhancing nanoparticle attachment to the negatively charged outer leaflet of the cellular plasma membrane, promoting nanoparticle uptake by endocytosis. In order to characterize extent and mechanism of cellular nanoparticle uptake, chitosan and the SPIO nanoparticle surfaces were fluorescently labeled with reactive Alexa Fluor 555 and 633 dyes (Invitrogen, USA), respectively, for multicolor tracking of particles. Intracellularly, chitosan may be cleaved from the particle surface, and so fate of the surface-attached chitosan and contrast-enhancing nanoparticles is tracked by time-lapse, confocal fluorescence imaging of cells. Particle fluorescence was endosome-compartmentalized during the first hour of uptake; however, evolution of cytosolic fluorescence was observed in following hours, suggesting endosomal escape of particles, as previously described for DNA/chitosan polyplex-gene carriers2, indicating a significant advantage for *in vitro* labeling of cells for non-invasive, cellular tracking *in vivo*.

References:

1. Wu, Y.L., et al., *PNAS,* **7**:103 (2006).
2. Roy, K., at al., *Nat. Med.*, **5**:4 (1999).

Niche-Mediated Control of Human Embryonic Stem Cell Self-Renewal and Differentiation

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Complexity in the spatial organization of human embryonic stem cell (hESC) cultures creates heterogeneous microenvironments (niches) that influence hESC fate. This study demonstrates that the rate and trajectory of hESC differentiation can be controlled by engineering hESC niche properties. Niche size and composition regulate the balance between differentiation-inducing and -inhibiting factors. Mechanistically, a niche size-dependent spatial gradient of Smad1 signaling is generated as a result of antagonistic interactions between hESCs and hESC-derived extra-embryonic endoderm (ExE). These interactions are mediated by the localized secretion of bone morphogenetic protein-2 (BMP2) by ExE and its antagonist, growth differentiation factor-3 (GDF3) by hESCs. Micropatterning of hESCs treated with small interfering (si) RNA against GDF3, BMP2 and Smad1, as well treatments with a Rho-associated kinase (ROCK) inhibitor demonstrate that independent control of Smad1 activation can rescue the colony size-dependent differentiation of hESCs. Our results illustrate, for the first time, a role for Smad1 in the integration of spatial information and in the niche-size-dependent control of hESC self-renewal and differentiation.

Propagation of Embryonic Stem Cells as Pluripotent Aggregates in Stirred-Suspension Vessels without Serum

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Recent advances in the field of embryonic stem cells (ESCs) have fueled great hope for regenerative and cell replacement therapies. Current culture methods of stem cell propagation and directed differentiation are impractical for meeting clinical needs. Alternative culture systems which are amenable to scale-up, have simple design, and allow for rigorous control of culture conditions are highly desirable. Previously, the use of stirred suspension bioreactors has been explored for the expansion of undifferentiated ESCs as aggregates. Here, self-renewing ESCs were propagated in such bioreactors with defined serum-free medium (DSFM). The presence of serum in the culture medium increases the variability in composition, the overall cost, and the risk for contamination with animal-derived pathogens.

Initially, a protocol was developed for adapting the culture of mESCs in dishes to serum-free conditions. Subsequently, mESCs in static culture with DSFM were transferred to stirred suspension vessels (100-250 ml) and in the same serum-free medium. Cells propagated more than 20-fold in 4 days while their viability was above 85%. Similar to mESC aggregates grown in serum-containing medium, dependence was observed of the average aggregate size of mESCs grown in DSFM on the agitation rate. Over multiple, successive passages in stirred suspension with DSFM the cells did not display gross karyotypic differences compared to ESCs maintained in dishes. Furthermore, the presence of stem cell markers such as Oct-4, Nanog and SSEA-1 was probed by quantitative PCR (qPCR), immunofluorescence imaging and flow cytometry. The expression of pluripotency genes/proteins in mESCs grown in stirred suspension without serum was similar to that of cells from dishes with DSFM, and static or suspension controls with serum. Cells were also subjected to *in vitro* differentiation to investigate whether their ability to give rise to progeny of the three germ layers was preserved. Differentiation towards cardiac mesoderm of mESCs from a bioreactor with DSFM resulted in beating cell clusters responding to pharmacological agents in an organotypic manner. Also, coaxing mESCs along the neuroectoderm lineage resulted in cells expressing β3-tubulin or glial fibrilar acidic protein (GFAP) whereas directed differentiation to definitive endoderm became apparent by the presence of Sox17+/Foxa2+ cells.

Based on these findings, the expansion of uncommitted ESCs in suspension bioreactors and in the absence of serum is feasible. Serum-free culture systems accommodating the self-renewal and directed differentiation of ESCs in a large scale will play an important role in regenerative medicine and stem cell biotechnology.

Protein Coated Scaffold for the Differentiation of Type II Pneumocytes from Murine Embryonic Stem Cells

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In tissue engineering, the cell-material interface plays a crucial role in cell function and proliferation. In order to engineer the gas-exchange component of the lung, we can grow type II pneumocytes and their derivatives, type I pneumocytes, on biodegradable scaffolds. We aimed to test the effects of different extracellular matrix proteins coated on tissue culture plastic and poly(D,L-lactide) (PDLLA) film on the differentiation and maintenance of type II pneumocytes derived from murine embryonic stem cells (mESC). A three-step differentiation protocol was applied to mESC transfected with a SPC-GFP hybrid gene, grown on collagen I, laminin 332, fibronectin, Matrigel and, as an inert control, gelatin. Higher levels of SPC mRNA expression and a greater percentage of SPC-eGFP-positive cells were seen following differentiation of mESC on Matrigel- or laminin 332-coated culture plates, respectively. The effects of the laminin 332 and Matrigel coating along with gelatin control were further evaluated on PDLLA film. Increased SPC mRNA expression was obtained for mESC differentiated on laminin 332-coated PDLLA film. Change in contact angle and æ potential of protein-coated tissue culture plastic and poly(D,L-lactide) film confirmed protein adsorption in each case. A huge decrease in contact angle of Laminin 332-coated PDLLA film corresponds to the increased SPC expression of type II pneumocytes, suggesting an chemical effect of the surface coating on pulmonary differentiation culture. It is concluded that laminin 332 and PDLLA provide a coating-scaffold combination that is suitable for engineering of distal lung tissue.

Reversible Biomolecule Self-Assembly and Presentation on Biomaterials Surfaces

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Addition of biomolecules to polymeric surfaces to control biocompatibility, cell attachment, and proliferation has been examined for

decades. Recently such surfaces have also been investigated with stem cell cultures, with the intention of addressing differentiation as well. Current methods for introducing biomolecule function have been either through bulk incorporation and diffusional release or through chemical conjugation. Chemical conjugation creates a surface which is time invariant, while diffusional release has complex temporal profiles which are limited due to loading capacity. Unfortunately stem cells require persistent microenvironments which predictably change over time, causing attachment and proliferation at one instance and differentiation at another.

In this study we examined the use of host-guest interactions to self-assemble peptides and proteins onto the surface of a biomaterial. These reversible interactions allow presentation of one biomolecule initially and replacement or refilling of another biomolecule when desired. β-cyclodextrin (CD) has been widely used to form selective and reversible, high affinity host-guest interactions with small, hydrophobic molecules such as adamantane (Ad). In this study, we examine the potential of using the interactions formed between CD and Ad derivatives to selectively and reversibly bind bioactive molecules to biomaterial surfaces to control stem cell microenvironment.

Poly(ethylene-co-vinyl alcohol) (pEVOH) was modified to contain a 2-10% CD substitution. These polymers were then added to tissue culture environments either by solvent casting or formation of an interpenetrating polymer network. Formation of host guest complexes on these surfaces was verified using Ad-PEG-fluorescein and fluorescence microscopy.

Using the same CD-Ad interactions, bioactive surfaces were made containing either the cell attachment peptide RGD or the non-attaching control peptide RGE, and were characterized by MALDI. GFP labeled mouse embryonic stem cells were observed to attach and proliferate on RGD surfaces, while little cell attachment was observed on RGE or surfaces with only Ad-PEG. Change from one surface type to another was observed by flushing an existing surface with an excess of the Ad-PEG version containing the other peptide. Similarly Epidermal Growth Factor (EGF) could be attached to the surface for bioactive function, which includes induction of cell proliferation, migration and differentiation in stem cells. Future directions include examining more complex biomolecules, such as bFGF and VEGF which our group is examining due to their capacity to induce cardiomyocyte and endothelial differentiation. In conclusion, we have demonstrated a new method for reversibly attaching molecules to biomaterial surfaces using CD-Ad interactions. Initial studies demonstrated reversible attachment of fluorescent molecules, bioactive peptides and simple proteins. Future research will examine how these surfaces can be used to examine how spatial and temporal arrangements of biomolecules can influence stem cell proliferation, migration and differentiation.

Role of p27 in Cyclic Adenosine Monophosphate Caused Differentiation in Rat Mesenchymal Stem Cells

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Mesenchymal stem cells (MSCs) have received special attention in stem cell engineering because of their multi potency. Thus, many efforts have been focused on how to control the growth and differentiation of MSCs. Recently, it was unveiled that chemical factors as well as physical factors, such as topography or stiffness of substrates influence on their growth, morphologies and differentiation. However, the mechanism explaining these changes has been not clearly presented. In this study, we present that membrane cholesterol concentration greatly influence on the extent of elongation of cells grown on topographically patterned surfaces. For example, cells whose membrane cholesterol was depleted with 2.5mM Methyl- beta-cyclodextrin elongated three times more than cholesterol enriched cells on a 1 micron groove patterned surface. These results suggest that cholesterol level of MSCs is an important factor for decision of their morphology in differentiating lineages.

Selective Tenocyte Differentiation of Mesenchymal Stem Cells Using Bone Morphogenetic Protein-12

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Introduction. Mesenchymal stem cells (MSCs) can differentiate into cells forming multiple tissue types including bone, cartilage and tendon[1]. MSCs are considered especially useful for tissue engineering applications, e.g. producing tissues for autologous transplantation. They can be collected from tissues like bone marrow with reasonable ease and safety, then expanded and induced to differentiate *in vitro* by a variety of procedures. Use of MSCs for tendon tissue engineering, however, has been limited by lack of reliable methods to precisely control tenocyte differentiation. In particular, an inductive factor specific for tenocyte differentiation has yet to be clearly identified.

Bone Morphogenetic Protein (BMP)-12, a cytokine of TGF?/BMP superfamily[2], is a candidate inducer of tenocyte differentiation, since transfection of MSCs with BMP-12 resulted in tendon-like tissue formation *in vivo*[2,3] and *in vitro*[3,4]. In the present study, we tested the ability of exogenous BMP-12 to selectively induce tenocyte differentiation from MSCs *in vitro*, in order to assess the potential utility of this approach for engineering of tendon tissues. We also began to characterize the roles played by scleraxis (Scx) and tenomodulin (Tnmd), two transcriptional regulators considered phenotypic markers of tenocytes, during BMP-12-induced tenocyte differentiation.

Discussion. Tendon tissue engineering based on mesenchymal stem cells offers novel strategies to enhance tendon healing. In developing new mesenchymal stem cell-based tissue engineering protocols, two concerns are i) control of the phenotype of the stem cells to induce proper formation of the desired tissue, and ii) insuring that the stem cells retain enough proliferative capacity to form sufficient tissue for a desired purpose, e.g. surgical implantation. The ability of MSCs to form tendon in response to BMP-12 both *in vivo* and *in vitro* has been demonstrated[2-6]. However, the specificity of BMP-12 as an inducer of tendon differentiation was not clearly established. Moreover, the signaling pathways that mediate the differentiation of mesenchymal stem cells into tenocytes is poorly understood. These issues are of particular interest since most BMPs have been defined by their ability to induce differentiation of tissues other than tendon, especially bone and cartilage[10,11]. Our finding that BMP-12 suppressed both chondrogenic and osteogenic differentiation of MSCs while increasing the number of cells expressing tenocyte phenotypic markers (Scx and Tnmd) clearly indicates that BMP-12 exerts a definite preference for promoting tendon differentiation. In addition, despite reducing the number of presumably undifferentiated (i.e. Nst-expressing) stem cells, BMP-12 treatment sustained a substantial number of those cells while simultaneously inducing tenocyte differentiation. These results indicate that the *in vitro* conditions used in this study are a reasonable start-

ing point for further development and optimization of a system for engineering of tendon tissues from mesenchymal stem cells. Finally, our data confirm that Scx plays a crucial, early role in the tenocyte differentiation pathway[7], upstream of both Nst and Tnmd. Taken together, these findings suggest that the combination of BMP-12 and MSCs *in vitro* represents a useful model both for the development of systems for tendon engineering and for investigating the mechanisms of tenocyte differentiation.

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References:

1. Herzog, E.L. et al, *Blood,* **102**:3483–93 (2003).
2. Wolfman, N.M. et al, *J. Clin. Invest.,* **100**: 321–30 (1997).
3. Lou, J. et al, *J. Orthop. Res.,* **19**:1199-202 (2001).
4. Lou, J. et al, *Clin. Orthop. Rel. Res.,* **369**:333–9 (1999).
5. Wang, Q.W. et al, *J. Biosci. Bioengineer.,* **100**:418-22 (2005).
6. Fu, S.C. et al (2003). *Life Sciences* **72**:2965-74 (2003).
7. Schweitzer, R. et al, *Development,* **128**:3855-66 (2001).
8. Shukunami, C. et al, *Dev. Biol.,* **298**:234-47 (2006).
9. Kafienah, W. et al, *Stem Cells,* **24**:1113-1120 (2006).
10. Storm, E.E. et al, *Nature*, **368**:639-43 (1994).
11. Reddi, A.H., *Nat. Biotech.,* **16**:247-52 (1998).

Serum-Free Derivation and Expansion of Endothelial from Embryonic Stem Cells

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Vascular progenitor cells derived from stem cells could potentially lead to a variety of clinically relevant applications including cell-based therapies and tissue engineering. Previously, our laboratory developed methods for isolating purified proliferating populations of vascular endothelial cells (EC) from mouse embryonic stem cells (ESC) using Flk-1 positive sorted cells, VEGF supplementation, and a rigorous manual selection technique required for endothelial cell purification and expansion, exhibiting approximately 25 population doublings.

These cell culturing conditions, however; required high levels of fetal bovine serum (FBS). Since FBS composition can vary significantly from batch-to-batch, the reproducibility of some aspects of these experiments has been somewhat unreliable. Attempts for reproducibility of serum cultures leads to tiresome batch lot testing. Testing must then be repeated when the desired lot is exhausted. Development of a serum-free induction system allows more control over the stem cell microenvironment.

Serum-free maintenance of mouse ESC has been previously established, but efforts to develop serum-free cultures for vascular differentiation have been more difficult. Our lab is currently developing an induction medium in which Knockout Serum Replacement, VEGF, and BMP-4 are used in lieu of traditional FBS. Results indicate that it is possible to generate comparable percentages (30%) of Flk-1+ vascular progenitors without FBS supplementation, but the serum-free cultures proliferate at a rate 3X slower than cultures with FBS.

We have also investigated the final expansion medium for the Flk-1 positive cells to develop into mature endothelial cells. Preliminary results indicate the presence of VE-cadherin+ and PECAM-1+ cells from cells cultured post-Flk-1+ enrichment in a chemically defined EC medium. These studies allow the full derivation process of mature endothelial cells from embryonic stem cells under serum free conditions.

Synergistic Acceleration of Stem Cell Mediated Heart Valve Tissue Formation by Cyclic Flexure and Laminar Flow

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Statement of Purpose: Bone marrow-derived mesenchymal stem cells (BMSC) represent a potentially attractive cell source for use in cardiovascular tissue engineering. BMSC can be isolated from adult patients relatively non-invasively, and BMSC have a pluripotent differentiation potential. Sutherland et al. recently demonstrated that BMSC isolated from juvenile sheep can be used to fabricate tissue engineered heart valves (TEHV), and that these TEHV can successfully function in the pulmonary outflow tract of sheep for at least 8 months. We previously demonstrated that cyclic flexure can independently stimulate engineered heart valve tissue formation by vascular smooth muscle cells (SMC). Toward developing optimized bioreactor conditioning regimens for BMSC-seeded TEHV, in the current study we investigated the independent and coupled effects of cyclic flexure and laminar flow on BMSC-seeded nonwoven scaffolds.

Methods: Ovine BMSC were isolated from juvenile sheep by the method of Pittenger et al. 2, expanded *in vitro* in DMEM supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic (Gibco). BMSC were seeded onto nonwoven 50:50 blend poly(glycolic acid) and poly(L-lactic acid) scaffolds (PGA/PLLA) (Albany International, Mansfield, MA) at a density of ~17x106 cells/cm2 as described previously. Following 30 hours seeding, BMSC-seeded scaffolds were maintained in static culture for 4 days and then loaded into our novel flex-stretch-flow (FSF) bioreactor. The FSF bioreactor accommodates 24 rectangular tissue specimens (~ 25 x 7.5 x 1 mm), with the ability to apply cyclic flexure and stretch by a linear actuator and laminar flow by a magnetically-coupled paddlewheel. A total of 48 BMSC-seeded scaffold specimens were prepared for evaluation in this study. Two separate runs of the bioreactor were required to test all of the specimens. In the first run, BMSC-seeded scaffolds were loaded into the bioreactor under aseptic conditions and incubated under static (n=12) or cyclic flexure (n=12) conditions. Cyclic flexure was applied at a frequency of 1 Hz and change-in-curvature of 0.554 mm-1. In the second run, BMSC-seeded scaffolds were incubated under laminar flow (n=12) or laminar flow/cyclic flexure (n=12) conditions. Laminar flow was applied by setting the paddlewheel rotational velocity to the maximum value of 2000 RPM, which was shown to yield an average fluid shear stress of 1.1505 dyne/cm2. Specimens from each mechanical loading group were removed following 1 (n=6) and 3 (n=6) weeks. Following removal from the FSF bioreactor, specimens were characterized by effective stiffness (E) testing, DNA and extracellular matrix (ECM) assays, histology, immunohistochemistry, and scanning electron microscopy (SEM).

Results / Discussion: By 3 weeks, the average DNA concentrations had decreased from 1 week values to 3.1 °" 0.5 (-89%; p<0.001), 3.8 °" 0.6 (-75%; p<0.001), 35.1 °" 1.8 (-23%; p<0.05), and 47.0 °" 4.8 (-21%; N.S.) *f*γg/g wet weight in the static, flex, flow, and flex-flow groups, respectively. By 3 weeks, the average S-GAG concentrations had increased from 1 week values to 3636 °" 345 (+21%; N.S.), 5637 °" 897 (+102%; p<0.01), and 2268 °" 67 (+1%; N.S.) micro g/g wet weight in the static, flex, and flow groups, respectively, and had decreased to 1919 °" 54 (-23%; p<0.05) micro g/g wet weight in the flex-flow group. Collagen was not detected biochemically at 1 week in any specimen group. By 3 weeks, the average collagen concentrations measured were

422 °" 98, 530 °" 106, 498 °" 95 and 844 °" 278 micro g/g wet weight in the static, flex, flow, and flex-flow groups, respectively. SEM provided dramatic evidence for accelerated tissue formation in the flex-flow group corroborated by histology.

Conclusions: The primary mechanical stimuli experienced by TEHV in pulsatile flow bioreactors, cyclic flexure and laminar flow, synergistically accelerated BMSC-mediated tissue formation. The results of this study provide guidance for optimizing bioreactor conditioning regimens for BMSC-seeded TEHV.

The Effect of Cell Density and Hypoxia on Potency of Rodent Multipotent Adult Progenitor Cells (MAPCs)

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Multipotent adult progenitor cells (MAPCs), isolated from postnatal bone marrow of rat, mouse, swine and human, can be expanded *in vitro* without showing obvious senescence. They can give rise to cell types of mesoderm, endoderm, and ectoderm. Recent modification in their isolation by culturing under hypoxic condition (5% O_2) has yielded cells expressing high levels of the embryonic stem cell specific transcription factor Oct4, which is associated with greater potency (High Oct4 MAPCs). MAPCs have been maintained at low cell densities (100-300 cells/cm^2) in order to maintain their potency. We studied the effects of high cell density and low ambient oxygen level on potency of high Oct4 rat MAPCs. The growth rate was not changed. Potency of undifferentiated rMAPCs, as measured by Oct4 mRNA and protein expression levels, was shown to be unaffected by higher cell densities up to 5000 cells/cm^2. Increased cell density did not significantly affect the differentiation potential towards endothelium-like and hepatocyte-like cells as evaluated by mRNA levels of endothelial and hepatocyte markers. Changing ambient oxygen from 5% O_2 (under which MAPCs were isolated and cultured) to 21% O_2 did not interfere with the potency, Oct4 levels, and proliferation of high Oct4 expressing rMAPCs. The results provide evidences that high Oct4 rMAPCs can be readily cultured at higher cell density than previously thought. These findings can open the possibility of easy scale up for potential clinical application of human MAPCs.

Tracking of Oligodendrocyte Remyelinated Axons in Spinal Cords

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Demyelination, the loss of the myelin sheath that insulates axons, is a prominent feature in many neurological disorders including multiple sclerosis (MS) and spinal cord injury (SCI). As a result of demyelination, signals are disrupted within the axons, leading to the loss of motor and other functions. The myelin sheath can be replaced by a process called remyelination, which can be initiated by transplanting stem cell derived oligodendrocyte progenitor cells into the injury site. Animal models have shown the effectiveness of this treatment for partially restoring the myelin sheath and some of the motor function.

We describe a method for automated tracking of the fate of these progenitor cells. The method uses cross-sectional histology, high-resolution imaging, and newly developed digital image processing algorithms to distinguish between regular oligodendrocyte-myelinated axons, newly oligodendrocyte-remyelinated axons and Schwann cell-remyelinated axons, and to track these cell types over time. The method provides an accurate count for each cell type, which is consistent with results obtained from a domain expert by means of cross-verification.

The method, which is based on high-resolution microscopic imagery and multiple time series, can be used for temporal tracking of the amount of newly remyelinated axons after human embryonic stem cell (hESC) treatment.

The gradient-based progressive isocontouring for cell detection is a generic pre-processing method to detect closed shapes. We have developed a robust method for stem cell fate tracking based on a G-ratio classification.

Our success in 2D geometry based post-processing of structures for axon-separation and noise removal might evoke great interest in the newly developing bio-geometry community. This automated classification of oligodendrocyte-remyelinated axons has also injected a lot of excitement among the neurobiologist collaborators. This project will relieve them of several weeks of pain-staking routine, repetitive, and mundane task that consumes several hours of trained people power. The accuracy of our detection and classification results have been corroborated, appreciated, and accepted by them and on account of its reliability, we are sure these methods will find widespread applicability reducing the turnaround time of their research.

Transcriptional Profiling to Understand Genomic Instability during Human Embryonic Stem Cell Propagation

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Human embryonic stem cells (hESCs) have generated a lot of interest in the scientific community based on their dual properties of limitless self-renewal and the pluripotent potential to differentiate into multilineage tissues. These unique properties have contributed to the widespread hope that many debilitating diseases might be cured or treated through stem cell applications. However, there is a critical need for a thorough characterization of the behavior of hESCs prior to realization of their use in clinical settings. Most of the focus on cell-fate decisions associated with hESCs revolves around self-renewal, differentiation and/or apoptosis. An often-overlooked cell fate is chromosomal instability in short-term lab cultures, which can present a major hurdle in safely and effectively implementing hESCs in clinical trials or cellular therapies. We have earlier demonstrated that hESCs are subject to chromosomal aberrations in a passaging-dependent manner. Furthermore, we have found that chromosomal alterations in hESCs changes their transcriptome, suggesting that directed-differentiation is likely to be affected. Using Affymetrix datasets obtained from different normal and abnormal samples from nine different hESCs, we report that abnormal hESCs have a genomic signature characterized by enrichment of genes located on specific chromosomes. Differential gene expression analyses resulted in many candidate genes for further analysis with the enriched genes in abnormal hESCs directly correlating with known trisomies.

The transcriptional profile of and the chromosomal copy number changes in abnormal hESCs serve as genomic landmarks to further identify key self-renewal regulators and proliferative mechanisms that provide abnormal hESCs selective growth advantage over normal hESCs. Our findings have important implications for hESCs to reach their full potential in regenerative medicine, as it is critical to address the mechanisms that could lead to genomic instability to develop appropriate propagation strategies for generation of chromosomally stable hESCs.

Tuning Hydrogel Modulus and Ligand Density Independently to Control Adult Neural Stem Cell Self-Renewal and Differentiation

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Stem cells are defined by their capacities for self-renewal and differentiation into one or more cell lineages. Without tight regulation or control of these properties; however, any derivative cell population will exhibit a range of heterogeneous phenotypes, yielding artifacts that may complicate the development of pharmaceuticals and cell therapies. Recent work demonstrates that biomaterials (i.e., matrices, scaffolds, culture substrates) can present key regulatory signals that combine with other environmental and genetic influences to create synthetic microenvironments that control stem cell fate. It can be argued that many of the promising therapeutic applications of stem cells will require instructive materials that exert active control over stem cell phenotype. Such materials may be designed for stem cell expansion and differentiation ex vivo, tissue regeneration via implantation with stem cells, or implantation alone to direct endogenous stem cell behavior. This work addresses the design of such synthetic materials, namely the design of material modulus and ligand density to control adult neural stem cell self-renewal and differentiation. We exploit the physical and chemical properties of hydrogels (polymers containing a significant volume of water) to mimic the native extracellular matrix surrounding mammalian cells. Using a biomimetic hydrogel, we define a robust synthetic and fully mechanically and chemically defined platform to regulate stem cell number and differentiation for the culture of adult neural stem cells. The synthetic hydrogel material properties, such as ligand type, ligand surface density, and stiffness (i.e., complex modulus), are quantitatively controlled and characterized. Previous work indicated that hydrogels, modified with the cell-binding ligand CGGNGEPRGDTYRAY from bone sialoprotein [bsp-RGD(15)] and synthesized on top of a stiff polystyrene substrate, can be used to regulate stem cell self-renewal and differentiation in a dose-dependent manner. To mimic the mechanical properties of functional tissue, we have synthesized hydrogels with bsp-RGD(15) with elastic moduli (i.e., stiffness) of 10-1 to 102 kPa. At low elastic moduli (~1 kPa), the elastic modulus significantly effects self-renewal and differentiation of adult hippocampal neural stem cells. Ultimately, one can define a phenotype or proliferation rate of a neural stem cell culture by tuning the material properties of the hydrogel culture material.

Ultra-High-Throughput Production of Highly Organized and Reproducible Human Embryoid Bodies

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Human embryonic stem cells (hESC) provide a window into early human development, and a renewable source of cells for regenerative medicine. However, the embryoid bodies (hEB) typically employed to reveal hESC developmental potential are heterogeneous and exhibit disorganized and inefficient differentiation. We therefore developed a "Spin-In Spin-Out" method to form large numbers of well-defined aggregates exhibiting multi-lineage differentiation and substantially improved self-organization from single-cell suspensions in well plates. These aggregates exhibit coordinated bi-domain structures including contiguous regions of extraembryonic endoderm- and epiblast-like tissue. In order to further increase throughput, silicon-wafer microfabrication techniques were then used to generate surfaces tiled with hundreds to thousands of microwells per square centimeter. Over 50,000 aggregates have been produced in a single trial using this system, with further increases limited primarily by the availability of cultured hESC. We thus present a scalable, ultra high throughput (UHTP) approach for generating spatially and temporally synchronized hEB that exhibit aspects of peri-implantation tissue-level morphogenesis. These results will advance fundamental studies into early human developmental processes, enable high-throughput screening strategies to identify conditions that specify hESC-derived cells and tissues, and directly transition the output of such work to pre-clinical production scales.

SBE's Ist International Conference on Stem Cell Engineering is organized by the Society for Biological Engineering