Society for Biological Engineering
An AIChE Technological Community

ISSCR
International Society for Stem Cell Research

3rd International Conference on Stem Cell Engineering

"Designing Cellular Therapies"

April 29 – May 2, 2012 • Seattle, WA, The Sheraton Hotel in Downtown Seattle

Society for Biological Engineering
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10:00 am - 10:20 am	Break
10:20 am - 12:35 pm	**Session 2:** Adult Stem Cell Isolation, Expansion, Differentiation and Bioprocessing
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5:20 pm - 7:00 pm	Poster Session with Refreshments
	Free Night for Dinner

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WELCOME ADDRESS

Greetings!

It is our pleasure to welcome you to Seattle and the Third International Conference on Stem Cell Engineering, co-sponsored by the Society for Biological Engineering and the International Society for Stem Cell Research. The theme of this meeting, "Designing Cellular Therapies", encompasses established and emerging areas of stem cell engineering that will need to interact to address the key challenges in stem cell biology and regenerative medicine.

Our goal is to facilitate integration of stem cell biology and engineering communities in academia and industry to catalyze a coordinated effort toward realizing the potential of stem cells. We feature recent advances with a forward looking perspective that identify and address challenges in commercial and medical use of stem cells for screening, disease modeling, and therapies. Our closing panel features an international assessment of stem cell engineering and discusses its opportunities and priorities.

In recent years, substantial progress has been made in culture systems for derivation, expansion, and differentiation of different stem cell types. Important challenges remain, including construction of physiologically relevant microenvironments to provide defined cues to cells in the appropriate spatial and temporal manner, robust scaling of culture and differentiation systems to produce sufficient numbers of cells for biomedical applications, determining molecular and phenotypic metrics to define stem cells and their progeny, and monitoring, understanding, and controlling heterogeneity in culture.

The emerging ability to reprogram cells from one state to another offers tremendous potential for generating stem cells and somatic cells with a desired genetic background, including patient-specific cells and models of genetic diseases. Stem cell engineering will contribute to the understanding of cell control mechanisms that define cell state and the development of tools to make reprogramming processes more robust and efficient.

Moving stem cells to the clinic will require expertise in tissue engineering, including constructing microenvironments to support cells, guide their function, and facilitate delivery. Understanding and regulating the dynamic interplay between stem cells and the body during processes such as vascularization and immune interactions will be critical for obtaining effective outcomes. Stem cells provide unique opportunities for addressing these chronic challenges in tissue engineering based on their ability to regenerate tissues comprised of multiple cell types *in vivo*.

We gratefully acknowledge the contributions of the many colleagues who have made this meeting possible. We thank the Society for Biological Engineering (SBE) for initiating, organizing, and supporting the three International Conferences on Stem Cell Engineering. The International Society for Stem Cell Research (ISSCR) has been a close partner with SBE, and this relationship has contributed to the quality and growth of this conference. The Scientific Advisory Board provided valuable guidance to selecting the meeting's themes and speakers as well as logistical aspects. We thank the session chairs, speakers and poster presenters, and other participants for sharing their work and ideas with the community. Finally, this meeting could not happen without the generous financial support of the National Science Foundation and our not-for-profit and corporate sponsors.

During the next few days, we hope you'll be inspired by the speakers and poster presenters, and actively participate in discussions with colleagues and sponsors. Finally, we hope you take some time to enjoy Seattle. It is a beautiful city, rain or shine, with a wide variety of interesting restaurants, bars, coffee shops, theaters and funky artists, and you are within striking distance of two mountain ranges and four National Parks.

Thank you for your participation,

Chuck Murry
Chair
University of Washington

Sean Palecek
Chair
University of Wisconsin - Madison

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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.

SBE provides:

- An international professional network
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SBE is governed by a Managing Board of industrial and academic leaders, which sets the course for the society.

The International Society for Stem Cell Research (ISSCR) is an independent, nonprofit organization established to promote and foster the exchange and dissemination of information and ideas relating to stem cells, to encourage the general field of research involving stem cells and to promote professional and public education in all areas of stem cell research and application.

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ISSCR
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Sunday, April 29

5:30 pm - 6:30 pm	**Welcome Reception**	
6:30 pm - 10:00 pm	**Dinner**	
6:30 pm - 6:40 pm	**Introductory Remarks: Chuck Murry** *(University of Washington)* **and Sean Palecek** *(University of Wisconsin)*	
6:40 pm - 7:25 pm	**Keynote Presentation: Gordon Keller**	

Monday, April 30

8:30 am - 10:00 am	**Session 1: Pluripotent Stem Cell Expansion, Differentiation and Bioprocessing** **Chairs: Bill Miller** *(Northwestern University)* **and E. Tzanakakis** *(State University of New York at Buffalo)*	
8:30 am - 9:00 am	Steve Oh *(Bioprocessing Technology Institute)*	Stem Cell Bioprocessing: Increasing Efficiency and Integrating Cell Expansion with Differentiation on Microcarriers
9:00 am - 9:20 am	Todd McDevitt *(Georgia Institute of Technology)*	Engineering 3D Pluripotent Stem Cell Aggregates for Scalable Directed Differentiation
9:20 am - 9:40 am	Taby Ahsan *(Tulane University)*	ROCK-Myosin II Regulation of Embryonic Stem Cell Differentiation
9:40 am - 10:00 am	Kathryn C. Davidson *(University of Washington School of Medicine)*	Wntß-Catenin Signaling Promotes Differentiation, Not Self-Renewal, of Human Embryonic Stem Cells and Is Repressed
10:00 am - 10:20 am	**Break**	
10:20 am - 11:50 am	**Session 2: Adult Stem Cell Isolation, Expansion, Differentiation and Bioprocessing** **Chairs: Jon Rowley** *(Lonza)* **and Johnna Temenoff** *(Georgia Institute of Technology)*	
10:20 am - 10:50 am	Irwin Bernstein *(Fred Hutchinson Cancer Research Center)*	Notch-Mediated Regulation Of Hematopoiesis: Basic And Clinical Implications
10:50 am - 11:10 am	Joaquim M.S. Cabral *(Instituto Superior Técnico, Technical University of Lisbon)*	Bioengineering Approaches for the Optimization of the Ex-Vivo Expansion of Hematopoietic Stem/Progenitor Cells for Cell Therapy
11:10 am - 11:30 am	Chris J. Hewitt *(Loughborough University)*	Larger Scale Expansion of Human Mesenchymal Stem Cells On Microcarriers
11:30 am - 11:50 am	Lisa Flanagan *(University of California, Irvine)*	Isolation of Specific Progenitor Cells with Label-Free DEP Sorting
11:50 pm - 12:35 pm	**Keynote Presentation: Peter Zandstra**	Feedback Control of Endogenous Signaling to Guide Stem Cell Fate
12:35 pm - 1:40 pm	**Lunch**	
1:40 pm - 3:10 pm	**Session 3: Engineering Environments to Control Cell Fate** **Chairs: Andre Levchenko** *(Johns Hopkins University)* **and Joel Voldman** *(Massachusetts Institute of Technology)*	
1:40 pm - 2:10 pm	Bill Murphy *(University of Wisconsin Stem Cell and Regenerative Medicine Center)*	Harnessing Endogenous Signals in the Stem Cell Microenvironment
2:10 pm - 2:30 pm	Adam Engler *(University of California, San Diego)*	Diblock Copolymer Foams with Adhesive Nano-Domains Promote Stem Cell Differentiation
2:30 pm - 2:50 pm	Cynthia Reinhart-King *(Cornell University)*	Micromolded Hydrogel Substrates of Tunable Stiffness
2:50 pm - 3:10 pm	Tracy Ooi *(The University of Texas at Austin)*	Generating Functional T Cells From Stem Cells: On-Demand and Renewable Cell Immunotherapy
3:10 pm - 3:30 pm	**Break**	
3:30 pm - 5:20 pm	**Session 4: Stem Cell Models of Development and Disease** **Chairs: Taby Ahsan** *(Tulane University)* **and Adam Engler** *(University of California, San Diego)*	
3:30 pm - 4:00 pm	Fionna Watt *(University of Cambridge)*	Stem Cell Behavior as Controlled by Intrinsic Mechanisms
4:00 pm - 4:20 pm	Nicola Elvassore *(Venetian Institute of Molecular Medicine)*	Dystrophin Expression Restoration by Human Artificial Chromosome In Cardiomyocytes Derived From DMD Patient hiPS Cells
4:20 pm - 4:40 pm	Yubing Xie *(State University of New York at Albany)*	Bioengineering Embryonic Stem Cell-Breast Cancer Cell Interactions to Understand Metastasis
4:40 pm - 5:00 pm	Rao Balaji *(North Carolina State University)*	Pluripotent Stem Cell Derived Models of Human Trophoblast Differentiation *in vivo*
5:00 pm - 5:20 pm	Roger A Pedersen *(Cambridge Stem Cell Institute)*	Mesoderm Differentiation and Patterning in Human Embryonic Stem Cells
5:20 pm - 7:00 pm	**Poster Session with Refreshments** **Free Night for Dinner**	

Tuesday, May 1

8:30 am - 10:00 am	**Session 5: Cellular Reprogramming** **Chairs: Balaji Rao** *(North Carolina State University)* **and David Schaffer** *(University of California, Berkeley)*	
8:30 am - 9:00 am	Joe Wu *(Stanford School of Medicine)*	Human Induced Pluripotent Stem Cells
9:00 am - 9:20 am	Tyler Gibson *(Duke University)*	Single-Cell Dynamics of Cell Lineage Commitment
9:20 am - 9:40 am	Eytan Abraham *(Pluristem Therapeutics)*	3D Cultured Placenta Derived MSC-like Cells for Cell Therapy
9:40 am - 10:00 am	Laertis Ikonomou *(Boston University)*	Efficient Derivation of Purified Nkx2-1+ Lung and Thyroid Progenitors Via Directed Differentiation of Mouse ES Cells
10:00 am - 10:20 am	**Break**	

10:20 am - 11:50 am	**Session 6: Vascularization** **Chairs: Kara McCloskey** *(University of California, Merced)* **and Ying Zheng** *(University of Washington)*	
10:20 am - 10:50 am	Jalees Rehman *(University of Illinois, Chicago)*	
10:50 am - 11:10 am	Kara McCloskey *(University of California, Merced)*	Specialized Tip- and Phalanx-Like Endothelial Cells Derived From Embryonic Stem Cells
11:10 am - 11:30 am	Warren Grayson *(Johns Hopkins University)*	Vascular Morphogenesis of Adipose-Derived Stem Cells Is Mediated by Heterotypic Cell-Cell Interactions
11:30 am - 11:50 am	Sharon Gerecht *(Johns Hopkins University)*	Regulating the Formation of 3D Vascular Networks
11:50 am - 12:35 pm	**Keynote Presentation: Robert Deans**	
12:35 pm - 1:40 pm	**Lunch**	
1:40 pm - 3:10 pm	**Session 7: Stem Cells in Tissue Engineering and Regenerative Medicine** **Chairs: Todd McDevitt** *(Georgia Institute of Technology)* **and Cynthia Reinhart-King** *(Cornell University)*	
1:40 pm - 2:10 pm	Buddy Ratner *(University of Washington)*	Human Embryonic Stem Cells and an Engineering Approach to Heart Repair
2:10 pm - 2:30 pm	Brendan Harley *(University of Illinois at Urbana-Champaign)*	Patterning Biochemical and Structural Cues Into Collagen-GAG Scaffolds to Alter Mesenchymal Stem Cell Bioactivity for Tendon Insertion Regeneration
2:30 pm - 2:50 pm	Manolis Tzanakakis *(State University of New York at Buffalo)*	Expansion and Differentiation of Human Pluripotent Stem Cells In Stirred-Suspension Microcarrier Culture
2:50 pm - 3:10 pm	Katsuhisa Matsuura *(Tokyo Women's Medical University)*	Creation of Cell Sheet-Based Bioengineered Heart Tissue Using ES/IPS Cells-Derived Cells-Derived Cells
3:10 pm - 3:30 pm	**Break**	
3:30 pm - 5:00 pm	**Session 8: Immunity, Inflammation, and Fibrosis** **Chairs: Ed Botchwey** *(University of Virginia)* **and Sharon Gerecht** *(Johns Hopkins University)*	
3:30 pm - 4:00 pm	Themis Kyriakides *(Yale School of Medicine)*	Identification of Molecular Modulators of Cell-Biomaterial Interactions
4:00 pm - 4:20 pm	Kareen L. Kreutziger *(University of Washington)*	Host Response and Vascularization of hESC-Derived Engineered Cardiac Tissue In a Rat Model of Myocardial Infarction
4:20 pm - 4:40 pm	Bill Miller *(Northwestern University)*	Depletion of SIRT1, but Not SIRT2, Inhibits PMA-Stimulated Megakaryocytic Differentiation of the K562 Cell Line
4:40 pm - 5:00 pm	Ying Zheng *(University of Washington)*	Microstructural Controls to Guide Tissue Growth in vitro and *in vivo*
5:00 pm - 7:00 pm	**Poster Session with Refreshments** **Free Night for Dinner**	
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8:00 am - 9:30 am	**Session 9: Clinical Translation of Cellular Therapies** **Chairs: Jennifer Elisseeff** *(Johns Hopkins University)* **and Jane Lebkowski**	
8:00 am - 8:30 am	Michael Laflamme *(University of Washington)*	Human Embryonic Stem Cell-Derived Cardiomyocytes Couple with Host Myocardium and Reduce Arrhythmias in a Guinea Pig Infarct Model
8:30 am - 8:50 am	Charles A. Gersbach *(Duke University)*	Correction of Duchenne Muscular Dystrophy by Engineered Nucleases
8:50 am - 9:10 am	Cecilia M. Giachelli *(University of Washington)*	Engineered Monocytes As a Cell Therapy for Ectopic Calcification
9:10 am - 9:30 am	Sarah Pringle *(University Medical Centre Groningen)*	Salivary Gland Stem Cell Therapy for Radiation-Induced Hyposalivation
9:30 am - 9:50 am	**Break**	
9:50 am - 11:50 am	**Session 10: Technologies for Stem Cell Analysis and Characterization** **Chairs: Ali Khademhosseini** *(Harvard University)* **and Shyni Varghese** *(University of California, San Diego)*	
10:50 am - 11:10 am	Johnna Temenoff *(Georgia Institute of Technology)*	Hydrogel Platforms to Examine Cell-Cell Signaling In MSC Differentiation
11:10 am - 11:30 am	Elisa Cimetta *(Columbia University)*	Spatial and Temporal Regulation of Signaling Pathways In hESCs Using Microfluidic Technologies
11:30 am - 11:50 am	Adina Scott *(University of Washington)*	An Automated Microfluidic Stem Cell Bioreactor
11:50 am - 12:35 pm	**Keynote Presentation: Jeanne Loring**	The Secret Lives of Stem Cells: Genomics and Epigenetics of Human Pluripotent Stem Cells
12:35 pm - 1:35 pm	**Panel Discussion with: Chuck Murry, Bob Nerem, Jon Rowley, David Schaffer, and Joe Wu** **Closing Remarks: Chuck Murry and Sean Palecek** **Lunch**	
1:35 pm - 2:00 pm	**Awards**	

1. 3D Cultured Placenta Derived MSC-Like Cells for Cell Therapy – Into the Clinic

Eytan Abraham, *Maya Wadmany, Harel Kasuto and Ayelet Chajut*
Pluristem Therapeutics, Haifa, Israel

2. Adhesive Signature-Based, Label-Free Isolation of Human Pluripotent Stem Cells

Ankur Singh[1]*, Shalu Suri[2], Ted Lee1, Jamie Chilton[3], Steven Stice[4], Hang Lu[2], Todd McDevitt[4] and Andres Garcia[1]*
[1]Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA
[2]School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA
[3]ArunA Biomedical, Inc.
[4]Biomedical Engineeing, Georgia Institute of Technology, Atlanta, GA

3. Airbrushing: A Rapid, Low-Cost Method for Fabricating Polymeric Nanofiber Scaffolds for Tissue Engineering

Wojtek Tutak*, Sheng Lin-Gibson, Tanya Farooque, Jyotsnendu Giri, Dongbo Wang and Carl G. Simon[1]*
NIST, Gaithersburg, MD

4. Analysis of Cardiomyocyte Aggregates with Time Lapse Phase Contrast Microscopy

Liew Seaw Jia[1], *Sherwin Ting Qi Peng[2], Chong Wee Keat[1], Tham Jo Yew[3], Li Xiang[1], Steve Oh[2]*
[1]Singapore Institute of Manufacturing Technology
[2]Bioprocessing Technology Institute
[3]Institute for Infocomm Research

5. Analyzing the Spatiotemporal Dynamics of Pluripotent Stem Cell Differentiation In 3D Environments Via Rules-Based Computational Modeling

Douglas White[1]*, Melissa A. Kinney[1], Melissa Kemp[2] and Todd C. McDevitt[1-2]*
[1]The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA
[2]The Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA

6. Arteriogenic Growth Supports Vascularization of Cardiac Grafts

Jill Weyers[1]*, Elina Minami[2], Sarah Dupras[1], Kevin Weitz[1], Dara Carlson[1], Timothy Cox[3], Stephen Schwartz[1], Charles Murry[1,4] and William Mahoney Jr.[1]*
[1]Pathology, University of Washington, Seattle, WA
[2]Cardiology, University of Washington, Seattle, WA
[3]Pediatrics, Seattle Children's Research Institute, Seattle, WA
[4]Bioengineering, University of Washington, Seattle, WA

7. Benefits of Hypoxic Culture On Mesenchymal Stem Cells

Shih-Chieh Hung
Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan

8. Bioengineering Strategies for the Development of Robust and Integrated Processes for Expansion and Cryopreservation of Human Pluripotent Stem Cells

Margarida Serra[1,2], *Claudia Correia[1,2], Catarina Brito[1,2], Marcos F.Q. Sousa[1,2], Janne Jensen[3], Peter Bjorquist[3], Manuel J.T. Carrondo[1,4] and Paula M. Alves[1,2]*
[1]IBET, Oeiras, Portugal
[2]ITQB-UNL, Oeiras, Portugal
[3]Cellartis AB, Gothenburg, Sweden
[4]FCT/UNL, Monte da Caparica, Portugal

9. Biomimicry of Cellular Interactions in 3D Stem Cell Scaffolds Via Proteolipobead-Matrix Hybrid Systems

Lane Gilchrist[1]*, Bin He[2], Eric Fried[3], Jesse Martin[4]*
[1]Chemical Engineering and Biomedical Engineering, City College of New York of CUNY
[2]Department of Chemical Engineering, City College of New York (of CUNY), New York, NY
[3]Chemical Engineering, City College of New York of CUNY
[4]Biomedical Engineering, City College of New York of CUNY

10. Cell Line and Stage-Specific Optimization for Enhanced Endothelial Differentiation of Mouse and Human Embryonic Stem Cells

William Turner, *Drew E. Glaser, Sarah J. Parkhurst, Andrew B. Burns and Kara McCloskey*
School of Engineering, University of California, Merced, Merced, CA

11. Characterization of Thawed Human Hematopoietic and/or Mesenchymal Stem Cells Using Colony Forming Unit Assay

HyeonKyeong Lee[1], *SeungHoon Park[1], Su-Youne Han[1], JiHyang Kim[1], ChulGeun Kim[2] and Byung-Rok Do[1]*
[1]Biotechnology Research Institute, HurimBioCell Inc., Seoul, South Korea
[2]Dept of Life Science, Hanyang University, Seoul, South Korea

12. Characterization, Culture and Differentiation of NKX2.5 Derived Cardiac Precursor Cells From the Human Heart

Nuria Gago-Lopez[1,2]*, Obinna Awaji[2] and Robb Maclellan[1,3]*
[1]Cardiology, UW Medicine- South Lake Union, Seattle, WA
[2]Medicine/Cardiology, UCLA, Los Angeles, CA
[3]Cardiology, UW Medicine- Division of Cardiology, Seattle, WA

13. Controlled Generation of Hematopoietic Progenitor Cells From Pluripotent Stem Cells Using Microenvironmental Cues

Nafees Rahman[1,2]*, Kelly Purpura[3], Ryan Wylie[4], Molly S. Shoichet[5] and Peter W. Zandstra[3]*
[1]Chemical Engineering And Applied Chemistry, University of Toronto
[2]Institute of Biomaterials & Biomedical Engineering, University of Toronto
[3]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada
[4]Department of Chemistry, University of Toronto
[5]Donnelly Centre for Cellular and Biomolecular Research, Department of Chemical Engineering & Applied Chemistry, University of Toronto, Toronto, ON, Canada

14. A Convenient Micropillar-Based Surface to Control the Size and Distribution of Human Embryonic Cell Colonies

Pei-Lien Tseng[1]*, Shih-Han Hung[2], Tzu-Wen Chuang[1], Pei-Yi Lin[2], Hsan-Jan Yen[1], Shiaw-Min Hwang[2], Yi-Cheng Hsieh[1] and Ronald A. Faris[3]*
[1]Corning Research Center Taiwan, Corning Inc., Hsinchu, Taiwan,
[2]Food Industry Research and Development Institute, Hsinchu, Taiwan
[3]Corning Inc., Corning, NY

15. Culture with Human Platelet Lysate Enhances Mesenchymal Stem Cell Physiology

***Priya R. Baraniak[1]**, Sarah Griffiths[2], Ian B. Copland[3,4], Jacques Galipeau[5], Robert M. Nerem[1,4] and Todd C. McDevitt[1,4]*

[1]*The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA*

[2]*Parker H. Petit Institute of Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA*

[3]*Winship Cancer Institute, Emory University, Atlanta, GA*

[4]*Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA*

[5]*School of Medicine, Emory University, Atlanta, GA*

16. Cyclic Mechanical Stretch Affects Membrane Integrity During Myogenesis

***Federica Michielin**, Stefano Giulitti, Elena Serena and Nicola Elvassore*

Department of Industrial Engineering, University of Padova, Padova, Italy

17. Density Gradient Multilayer Polymerization: A Facile Method to Create Scaffolds for Culture of Complex Tissue

*Jerome Karpiak[1], and **Adah Almutairi[2]***

[1]*UC San Diego*

[2]*Pharmaceutical Sciences, University of California San Diego*

18. Design and Operation of A Bioreactor System for the Expansion of Mouse Embryonic Stem Cell-Derived Neural Stem Cells On Microcarriers

*Carlos A.V. Rodrigues, **Maria Margarida Diogo**, Cláudia Lobato da Silva and Joaquim M.S. Cabral*

Department of Bioengineering and Institute for Biotechnology and Bioengineering, Instituto Superior Técnico, Technical University of Lisbon, Lisboa, Portugal

19. Developing An in vitro Model of Ectopic Calcification to Test Osteoclastic Resorptive Activity

***Cameron W. Rementer,** Meiting Wu and Cecilia M. Giachelli*

Bioengineering, University of Washington, Seattle, WA

20. The Development of ES Cell Expansion System Using Hollow-Fiber Dialysis Membrane

***Ushio Iwamoto[1]**, Michi Sato[1], Kanako Konishi[1], Katsuhisa Matsuura[2], Masanori Wada[3], Hirotada Akashi[1], Mikitomo Yasutake[1], Tatsuya Shimizu[2] and Teruo Okano[2]*

[1]*Asahi Kasei Corporation, Tokyo, Japan*

[2]*Institute of Advanced Biomedical Engineering and Science (TWIns), Tokyo Women's Medical University, Tokyo, Japan*

[3]*ABLE Co., Tokyo, Japan*

21. The Development of Large Scale Continuous Perfusion Culture System for ES Cell Expansion and Cardiac Differentiation

***Masanori Wada[1]**, Katsuhisa Matsuura[2], Yoichi Ishikawa[1], Tatsuya Shimizu[2] and Teruo Okano[2]*

[1]*ABLE Co., Tokyo, Japan*

[2]*Institute of Advanced Biomedical Engineering and Science (TWIns), Tokyo Women's Medical University, Tokyo, Japan*

22. Dynamic Control Over the Mechanical Microenvironment During the Neuronal Differentiation of Mouse Embryonic Stem Cells

***Shahzad Ali[1]**, Andrew Pelling[2], Ivan Wall[1] and Farlan S. Veraitch[1]*

[1]*Biochemical Engineering, University College London, London, United Kingdom*

[2]*University of Ottowa*

23. Effect of Dissolved Oxygen Tension and Medium Exchange On the in vitro Proliferation and Metabolism of Human Mesenchymal Stem Cells: A Quantitative Approach

***Qasim A. Rafiq[1]**, Karen Coopman[1], Alvin W. Nienow[2], Christian van den Bos[3] and Christopher J. Hewitt[1]*

[1]*Chemical Engineering, Loughborough University, Loughborough, United Kingdom*

[2]*Birmingham University, Birmingham, UK*

[3]*LIFT, Lonza, Cologne, Germany*

24. Effects of Microencapsulated Osteoblast-Derived Growth Factors, Cell Ratio and Hydrogel Diameter On the *ex vivo* Expansion of Hematopoietic Stem/Progenitor Cells Under Hypoxia Environment

***Kedong Song,** Tianqing Liu, Xiangqin Li, Dan Ge and Hai Wang*

Dalian University of Technology, Dalian, China

25. Efficient Adenoviral Transduction In Stem Cells Through Cyclic Microfluidic-Assisted Infections At Low MOI

***Federica Michielin,** Camilla Luni, Stefano Giulitti and Nicola Elvassore*

Department of Industrial Engineering, University of Padova, Padova, Italy

26. Electrically Conductive, Biocompatible Composite Containing Carbon Nanobrushes for Applications in Neuroregeneration

***Erfan Soliman[1],** Sze C. Yang[2], George W. Dombi[2], and Sujata K. Bhatia[1]*

[1]*Harvard University, School of Engineering and Applied Sciences, Cambridge, Massachusetts*

[2]*University of Rhode Island, Department of Chemistry, Kingston, Rhode Island*

27. Endogenous Stem Cell Mobilization and Homing for Bone Regeneration Modulated by Sphingosine-1-Phosphate (S1P) Receptors

***Anusuya Das[1],** Anjan Kaushik[2], Anthony Awojoodu[1,3] and Edward Botchwey[3]*

[1]*Biomedical Engineering, University of Virginia, Charlottesville, VA*

[2]*Orthopaedic Surgery, UVA*

[3]*Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA*

28. Engineered Neural Tissue with Columns of Aligned Schwann Cell-Like Cells From Differentiated Adipose-Derived Stem Cells Can Support and Guide Neuronal Growth

***Melanie Georgiou[1],** Paul J. Kingham[2], Jon P. Golding[1], Jane Loughlin[1] and James B. Phillips[1]*

[1]*Faculty of Science, The Open University, London, United Kingdom*

[2]*Department of Integrative Medical Biology, Umeå University*

29. Engineering Cellular Homing and Migration to Enhance Immune and Stem Cell-Based Therapeutics

***Jason Park[1,2,3,4],** Faith R. Kreitzer[5] Benjamin Rhau[4], James Onuffer[3,4], Bruce R. Conklin[5] and Wendell A. Lim[4,6]*

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[2]*Gladstone Institute of Cardiovascular Disease, San Francisco, CA*

[3]*The Cell Propulsion Laboratory - an NIH Nanomedicine Development Center*

[4]*Department of Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, CA*

[5]*Gladstone Institute of Cardiovascular Disease, San Fransisco, CA*

[6]*University of California San Francisco, Howard Hughes Medical Institute*

30. Engineering of Human Bone Tissue From Pluripotent Stem Cells In Perfusion Bioreactors

Darja Marolt[1,2], *Ivan Marcos Campos[1], Sarindr Bhumiratana[1], Ana Koren[1,3], Petros Petridis[1], Geping Zhang[4], Patrice F Spitalnik[4], Warren Grayson[5] and Gordana Vunjak Novakovic[1]*
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[2]The New York Stem Cell Foundation, New York, NY
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[4]Department of Pathology and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, NY
[5]Department of Biomedical Engineering, School of Medicine, Johns Hopkins University, Baltimore, MD

31. Engineering the Microenvironment of the Adult Brain to Direct Neural Stem Cell Fate

Anthony Conway, *Randolph Ashton and David V. Schaffer*
Department of Chemical and Biomolecular Engineering, University of California, Berkeley, Berkeley, CA

32. Evaluation of Continuous, Scalable Concentration and Wash Systems for Cellular Therapies

Lauren DePalma, ***John Gaut,*** *Thomas Brieva, Li Ren and Greg Russoti*
Celgene Cellular Therapeutics

33. Expansion of Human Embryonic Stem Cells On Coating-Free Microcarriers In Serum Free Conditions

Allen Chen[1], *Xiaoli Chen[1], Andre Choo[1], Shaul Reuveny[1,2] and Steve K. W. Oh[1]*
*[1]Stem Cell Group, Bioprocessing Technology Institute A*STAR, Singapore*
[2]Department of Biotechnology, Israel Institute for Biological Research, Ness-Ziona, Israel

34. Expansion of Human Mesenchymal Stem Cells On Microcarriers Under Different Dissolved Oxygen Tensions

Qasim A. Rafiq[1], *Karen Coopman[1], Alvin W. Nienow[2], Christian van den Bos[3] and Christopher J. Hewitt[1]*
[1]Chemical Engineering, Loughborough University, Loughborough, United Kingdom
[2]Birmingham University, Birmingham, UK
[3]LIFT, Lonza, Cologne, Germany

35. Exposure of Mesenchymal Stem Cells to Electric Current Induces Osteodifferentiation

Courtney M. Creecy[1], Marissa E. Wechsler[1], Christine F. O'Neill[1], Bernard P. Arulanandam[2] and ***Rena Bizios[1]***
[1]Department of Biomedical Engineering, The University of Texas at San Antonio, San Antonio, TX
[2]Department of Biology, The University of Texas at San Antonio, San Antonio, TX

36. The Extracellular Matrix Is a Novel Attribute of Endothelial Progenitors and of Hypoxic Mature Endothelial Cells

Sravanti Kusuma[1], *Stephen Zhao[2], Bradley Isaacs[3] and Sharon Gerecht[2]*
[1]Biomedical Engineering, Johns Hopkins University, Baltimore, MD
[2]Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD
[3]Biomedical Engineering, Johns Hopkins University

37. Feeder-Free mRNA Reprogramming Methods for the Derivation of Clinically Relevant Human IPS Cell Lines

Kerry Mahon, *Charles Martin and Brad Hamilton*
Stemgent, Inc.

38. Fluid Shear Stress Pre-conditioning Enhances Embryonic Stem Cell Endothelial Differentiation under Low Oxygen Conditions

Barbara A. Nsiah[1,2], Robert M. Nerem[1,2,3], Todd C. McDevitt[2,3]
[1]George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA
[2]Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA
[3]Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA

39. Human Embryonic Stem Cell-Derived Cardiomyocytes Migrate In Response to Fibronectin and Wnt5a

Kara White Moyes[1], *Christopher Sip[2], Willimark Obenza[3], Emily Yang[3], Cody Horst[3], Robert Welikson[4], Stephen D Hauschka[4], Albert Folch[2] and Michael A Laflamme[5]*
[1]Pathology, University of Washington
[2]Bioengineering, University of Washington
[3]University of Washington
[4]Biochemistry, University of Washington
[5]Pathology, University of Washington, Seattle, WA

40. Human Pluripotent Stem Cell-Derived Cardiac Micro-Tissue Particles for Myocardial Infarct Repair

Kareen L. Kreutziger[1], *Kaytlyn Beres[2], Sarah Dupras[1], Sarah Fernandes[1], Xiulan Yang[1], Veronica Muskheli[1] and Charles Murry[1,2]*
[1]Pathology, University of Washington, Seattle, WA
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41. Image Analysis for Evaluation of Growth and Differentiation of Embryonic Stem Cells

Megan M. Hunt[1], *Guoliang Meng[2], Derrick E. Rancourt[2], Ian D. Gates[3] and Michael S. Kallos[1]*
[1]Pharmaceutical Production Research Facility (PPRF), Schulich School of Engineering, University of Calgary, Calgary, AB, Canada
[2]Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, Canada,
[3]Department of Chemical and Petroleum Engineering, Schulich School of Engineering, University of Calgary, Calgary, AB, Canada

42. Immunoaffinity Aqueous Two-Phase System Bioengineering Strategies for the Potential Recovery and Purification of Stem Cells

Mirna González-González and Marco Rito-Palomares
Centro de Biotecnología-FEMSA, Tecnológico de Monterrey. Campus Monterrey, Ave. Eugenio Garza Sada 2501 Sur, Monterrey, NL 64849, México

43. Inducible Fluorescence Exchange with Conditional Gene Over-Expression In Human Stem and Primary Cells by Zinc Finger Nuclease Mediated Genetic Engineering Differentiated Populations

Nathan Palpant, *Lil Pabon and Charles Murry*
Department of Pathology, University of Washington, Seattle, WA

44. Integrated Bioprocesses for Scalable Production, Purification and Cryopreservation of iPSC-Derived Cardiomyocytes

Margarida Serra[1,2], *Claudia Correia[1,2] Marcos F.Q. Sousa[1,2], Ana Teixeira[1,2], Catarina Brito[1,2], Karsten Burkert[3], Azra Fatima[3], Manuel J.T. Carrondo[2,4], Juergen Hescheler3, Tomo Saric[3] and Paula M. Alves[1,2]*

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[3]UKK, Cologne, Germany

[4]FCT/UNL, Monte da Caparica, Portugal

45. Inverse Agonism of Sphingosine 1-Phosphate Receptor Three Mobilized Hematopoietic Stem Cells with Long Term Engraftment Capability

Anthony Awojoodu[1,2], *Anusuya Das[1] and Edward Botchwey[2]*

[1]Biomedical Engineering, University of Virginia, Charlottesville, VA

[2]Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA

46. Isolation and Characterization of iPSC Using a Novel Alkaline Phosphatase Live Stain

Rene Quintanilla Jr.[1], *Candida Vaz[2], Elena Grigorenko[3], Vivek Tanavde[2] and Uma Lakshmipathy[1]*

[1]Primary and Stem Cell Systems, Life Technologies, Carlsbad, CA

*[2]Bioinformatics Institute, A*STAR, Singapore*

[3]NexGen qPCR, Life Technologies, Beverly, MA

47. Isolation and Mechanically-Induced Differentiation of Adipose-Derived Stem Cells to Improve Muscle Regeneration

Yu Suk Choi *and Adam J. Engler*

Bioengineering, University of California, San Diego, La Jolla, CA

48. Mesenchymal Stem Cell Gene, Protein and Epigenetic Response to Shear Stress Stimulus

Sarah Griffiths[1], *Yunzhe Zhang[2], Dylan S. Nass[1], Yuhong Fan[2] and Robert M. Nerem[1]*

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[2]School of Biology, Georgia Institute of Technology, Atlanta, GA

49. Microfluidic Control of Three Dimensional Embryonic Stem Cell Microenvironments

Melissa A. Kinney[1], *Rabbia Saeed[1], Boris Zakharin[1], Ari Glezer[2] and Todd C. McDevitt[1,3]*

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[2]The George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA

[3]The Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA

50. A Microfluidic Platform for hESC Differentiation Into Hepatocytes

Kevin Chen[1], ***Tzu-Wen Chuang[2],*** *Lih-Tao Hsu[1], Shiun-Yin Chang[1], Wannhsin Chen[1], Yi-Cheng Hsieh[2] and Ronald A. Faris[3]*

[1]Industrial Technology Research Institute, Hsinchu, Taiwan

[2]Corning Research Center Taiwan, Corning Inc., Hsinchu, Taiwan

[3]Corning Inc., Corning, NY

51. Microfluidic Single-Cell Analysis of Embryoid Body Heterogeneity Stem Cells

Jenna Wilson[1], *Shalu Suri[2], Hang Lu[2,3] and Todd McDevitt[1,3]*

[1]Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA

[2]School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA

[3]The Parker H. Petit Institute for Bioengineering and Bioscience, Atlanta, GA

52. Micropatterend Gel Controlled Tubulogenesis From Dispersed Ureteric Epithelial Cells

Peter V. Hauser[1,2], *Masaki Nishikawa[1,2], Hiroshi Kimura[3], Teruo Fujii[3] and Norimoto Yanagawa[1,2],*

[1]Renal Regeneration Laboratory, VAGLAHS at Sepulveda, North Hills, CA

[2]David Geffen School of Medicine, UCLA, Los Angeles, CA

[3]Institute of Industrial Science, University of Tokyo, Tokyo, Japan

53. Modeling a Human Genetic Disorder of the Autonomic Nervous System In Induced Pluripotent Stem Cells

Faith R. Kreitzer[1], *Jose Otero[2,3], Jason Park4, Mark J. Scott[1], Ethan W. Hua[1], Verenice Bravo[1], Alice Sheehan[1], Marie Sears[1], Po-Lin So[1], David Rowitch[3] and Bruce R. Conklin[1]*

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[2]Pathology, Division of Neuropathology, University of California San Francisco

[3]Pediatrics and Neurosurgery, University of California San Francisco

[4]Department of Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, CA

54. Modeling Congenital Central Hypoventilation Syndrome (CCHS) Using Induced Pluripotent Stem (iPS) Cells

Verenice Bravo[1], *Faith Kreitzer[1], Jose Otero[2,3], Po-Lin So[1], Ethan W. Hua[1,4], David Rowitch[4] and Bruce R. Conklin[1]*

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[2]Pathology, Division of Neuropathology, University of California San Francisco

[3]Pediatrics and Neurosurgery, University of California San Francisco

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55. Multifactorial Analysis of Embryonic Stem Cell Self-Renewal Reveals a Crucial Role of GSK-3-Mediated Signaling Under Hypoxia

Hélder S. C. Barbosa, ***Tiago G. Fernandes,*** *Tiago P. Dias, Maria Margarida Diogo and Joaquim M.S. Cabral*

Department of Bioengineering and Institute for Biotechnology and Bioengineering, Instituto Superior Técnico, Technical University of Lisbon, Lisboa, Portugal

56. Myosin-II Is a Central Node for Physical Regulation of Adult Hematopoiesis

Jae-Won Shin[1], *Amnon Buxboim[2] and Dennis E. Discher[1]*

[1]Chemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia, PA,

[2]University of Pennsylvania

57. Neural Crest-Derived Dental Pulp Stem Cells Function As Ectomesenchyme to Support Salivary Gland Tissue Formation

Kajohnkiart Janebodin

Oral Health Sciences, University of Washington, Seattle, WA

58. A Novel Method for Fabrication of Chemically Heterogeneous 3D Nanofibers Scaffold for Tissue Engineering Applications

Jyotsnendu Giri[1], *Marcus T. Cicerone[2] and Wojtek Tutak[3]*
[1]Polymer Division, National Institute of Standards and Technology, Gaithersburg, MD
[2]Polymers Division, Material Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, MD
[3]NIST, Gaithersburg, MD

59. Novel Strategies for 3D Neural Culture and Gene Delivery: Human Central Nervous System in vitro Models for Preclinical Research

Catarina Brito[1]*, Daniel Simão[1], Paulo Fernandes[1], Margarida Serra[1], Johannes Schwarz[2], Giampietro Schiavo[3], Eric J. Kremer[4] and Paula M. Alves[1]*
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[2]University of Leipzig, Leipzig, Germany
[3]Cancer Research UK, London, United Kingdom
[4]IGMM, Montpellier, France

60. A Novel Strategy to Genetically Engineer Neural Stem Cells

Catarina Madeira, *Mónica S.C. Reis, Filipa F.C.G. Ferreira, Carlos A.V. Rodrigues, Maria Margarida Diogo and Joaquim Cabral*
Department of Bioengineering and Institute for Biotechnology and Bioengineering, Instituto Superior Técnico, Technical University of Lisbon, Lisboa, Portugal

61. Qualification of 19F MRI to Detect CD34+ Hematopoietic Stem Cells *in vivo*

*Brooke Helfer[1], **Anthony Balducci[1]**, Zhina Sadeghi[2], Chris Flask[3], and Amy Wesa[1]*
[1]Research and Devleopment, Celsense, Inc, Pittsburgh, PA
[2]Urology, Case Western Reserve University, Cleveland, OH
[3]Radiology and Biomedical Engineering, Case Western Reserve University, Cleveland, OH

62. Optimisation of the Expansion and Differentiation of Embryonic Stem Cells On An Automated Microwell Platform

Nathalie Moens, *Waqar Hussain, Paul Mondragon-Teran, Diana Hernandez, Ludmila Ruban, Farlan Veraitch, Ivan Wall and Gary J. Lye*
Biochemical Engineering, University College London, London, United Kingdom

63. Possibility of in vitro Adipogenic Differentiation of Human Adipose-Derived Stem Cells Under Co-Culture Conditions In the Presence of Human Mature Adipocyte

Kedong Song, *Tianqing Liu, Dan Ge, Ruipeng Li, Shixiao Li, Yingchao Liu and Hai Wang*
Dalian University of Technology, Dalian, China

64. Potential for Hydrogel Composites Containing Carbon Nanobrushes as Stem Cell Scaffolds

William H. Marks[1], *Sze C. Yang[2], George W. Dombi[2], Sujata K. Bhatia[1]*
[1]Harvard University, School of Engineering and Applied Sciences, Cambridge, Massachusetts
[2]University of Rhode Island, Department of Chemistry, Kingston, Rhode Island

65. A Pre-Clinical Dataset Exploring the Effects of a 19F-MRI Cellular Contrast Agent on Dendritic Cells

*Brooke Helfer[1], **Anthony Balducci[1],** Julie Urban[2], Pawel Kalinsky[2] and Amy Wesa[1]*
[1]Research and Development, Celsense, Inc, Pittsburgh, PA
[2]Department of Surgery, University of Pittsburgh, School of Medicine, Pittsburgh, PA

66. Preparation of Homogeneous Progenitor Population From Human Umbilical Cord Tissue Using Gene Expression Profiling

Su-Youne Han[1], *Jung Ah Cho[1], JiHyang Kim[1], ChulGeun Kim[2] and Byung-Rok Do[1]*
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[2]Dept of Life Science, Hanyang University, Seoul, South Korea

67. Qualification of 19F MRI to Detect CD34+ Hematopoietic Stem Cells *in vivo*

*Brooke Helfer[1], **Anthony Balducci[1]**, Zhina Sadeghi[2] , Chris Flask[3], and Amy Wesa[1]*
[1]Research and Devleopment, Celsense, Inc, Pittsburgh, PA
[2]Urology, Case Western Reserve University, Cleveland, OH
[3]Radiology and Biomedical Engineering, Case Western Reserve University, Cleveland, OH

68. Regeneration of Peripheral Nerve by Newly Isolated Progenitors

Leili Ghazi Zadeh[1]*, Abbas Shafiee[1], Masoud Soleimani[2]*
[1]Stem Cell Biology Department, Stem Cell Technology Research Center, Tehran, Iran
[2]Department of Hematology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran

69. Ribonucleotide Reductase-Overexpressing Pluripotent Stem Cells As a Novel Inotropic Cardiac Therapy

Scott D Lundy[1], *Michael A Laflamme[2] and Michael Regnier[1]*
[1]Bioengineering, University of Washington
[2]Pathology, University of Washington, Seattle, WA

70. Robust Cardiomyocyte Differentiation From Human Pluripotent Stem Cells Via Temporal Modulation of Canonical Wnt Signaling

Xiaojun Lian[1], *Cheston H. Hsiao[1], Gisela Wilson[2], Kexian Zhu[1], Laurie Hazeltine[1], Samira M. Azarin[1], Kunil Raval[2], Jianhua Zhang[2], Timothy Kamp[2] and Sean P. Palecek[1]*
[1]Chemical & Biological Engineering, University of Wisconsin-Madison, Madison, WI
[2]School of Medicine, University of Wisconsin-Madison

71. A Scalable System for Production of Functional Pancreatic Progenitors From Human Embryonic Stem Cells

Thomas Schulz[1], *Holly Young[2], Alan Agulnick[2], Josie Babin[2], Emmanuel Baetge[2], Anne Bang[2], Anindita Bhoumik[2], Igor Cepa[2], Rosemary Cesario[2], Carl Haakmeester[2], Kuniko Kadoya[2], Jonathan Kelly[2], Justin Kerr[2], Larua Martinson[2], Amanda McLean[3], Mark Moorman[2], Janice Payne[2], Mike Richardson[2], Kelly Ross[2], Eric Sherrer[1], Xuehong Song[1], Alistair Wilson[2], Eugene Brandon[2], Chad Green[2], Evert Kroon[2], Olivia Kelly[2], Kevin D'Amour[2] and Allan Robins[1,2]*
[1]Viacyte, Athens, GA
[2]Viacyte, San Diego, CA
[3]Biochemistry and Molecular Biology, University of Georgia

72. Screening 3D Stem Cell Microenvironments

Adrian Ranga, *Samy Gobaa, Katarzyna Mosiewicz, Yuya Okawa, Andrea Negro and Matthias Lutolf*
EPFL, Lausanne, Switzerland

73. A Serum-Free and Xeno-Free Microcarrier-Based Scalable System for the Expansion of Human Mesenchymal Stem/Stromal Cells

Francisco dos Santos[1], Cláudia Lobato da Silva[1], Pedro Z. Andrade[1], Manuel M. Abecasis[2], Jeffrey Gimble[3], Andrew Campbell[4], Shayne Boucher[4], Eric Roos[4], Sandra Kuligowski[4], Lucas Chase[5], Mohan Vemuri[4] and ***Joaquim M.S. Cabral[1]***
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[2]IPOFG-Instituto Português de Oncologia Francisco Gentil, Lisboa, Portugal
[3]Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, LA
[4]Life Technologies, Corp., Carlsbad, CA,
[5]Cellular Dynamics International, Madison, WI

74. Targeted Insertion of a Selectable Floxed Dual Fluorescence Lineage Tracing Reporter In Human Pluripotent Stem Cells by Zinc Finger Nuclease

Jay Gantz[1], Nathan Palpant[2], *Charles Murry[2] and Michael A Laflamme[2]*
[1]Bioengineering, University of Washington, Seattle, WA
[2]Pathology, University of Washington, Seattle, WA

75. Temporal Application of Topography to Increase the Rate of Neural Differentiation From Human Embryonic Stem Cells

Lesley Y. Chan[1,2,3], *Evelyn KF Yim[2,4,5] and Andre Choo[1,2,3]*
[1]Stem Cell Group, Bioprocessing Technology Institute, Singapore, Singapore
[2]Department of Bioengineering, National University of Singapore, Singapore, Singapore
[3]NUS Graduate School for Integrative Sciences & Engineering, National University of Singapore, Singapore, Singapore
[4]Department of Surgery, National University of Singapore, Singapore, Singapore
[5]Mechanobiology Institute Singapore, National University of Singapore, Singapore, Singapore

76. Three-Dimensional Culture and Differentiation of Human Adipose Tissue-Derived Stromal Cells In Chitosan/α-Glycerophosphate/Collagen Hydrogels

Kedong Song*, Tianqing Liu, Dan Ge, Xiangqin Li and Hai Wang,*
Dalian University of Technology, Dalian, China

77. Three-Dimensional Culture of Neural STEM/Progenitor CELLS/COLLAGEN Sponge Constructs IN Perfusion Bioreactor

Tianqing Liu, *Dan Ge, Kedong Song, Guan Shui and Ma Xuehu*
Dalian University of Technology, Dalian, China

78. Two Human Embryonic Stem Cell Derived Clonal MSC Cell Lines Showed Distinct Proliferation and Differentiation Properties

Amer Mahmood
College of Medicine, King Saud University, Riyadh, Saudi Arabia

79. Using Electrospun Poly (e-caprolactone) Nanofibers to Promote the Differentiation of Induced Pluripotent Stem Cells Into Neural Phenotypes

Nima Khadem Mohtaram[1]*, Stephanie Morison[1], Junghyuk Ko[1], Martin Byung-Guk Jun[1] and Stephanie Willerth[1,2]*
[1]Mechanical Engineering, University of Victoria, Victoria, BC, Canada
[2]Division of Medical Sciences, University of Victoria, Victoria, BC, Canada

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San Juan, Puerto Rico
The Sheraton Old San Juan Hotel

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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. It is the job of the speakers and session chairs to ensure that all talks are ready for presentation. Speakers will have 15 minutes for their talk, including questions. Please help us remain on time.

POSTER PRESENTERS

Please set up your poster in the Grand Ballroom A/B Rooms before the reception. Posters may be left up until Wednesday morning, 9:00 am, May 2nd.

IMPORTANT ADDRESSES

SBE staff will be available at the Registration Booth in the Prefunction Area outside of the Grand Ballroom at the following times:

Sunday, April 29	3 pm - 7 pm
Monday, April 30	7 am - 12 pm
Tuesday, May 1	7 am - 12 pm
Wednesday, May 2	7 am - 12 pm

Following the conference, you may reach SBE's Technology Associate, Derek Lapiska, by email at bio@aiche.org or by phone at 646-495-1381.

Sunday Evening Dinner and Reception

Keynote Presentation

Gordon Keller

Dr. Gordon Keller earned his PhD in Immunology at the University of Alberta in 1979 and completed a Post Doctoral Fellowship at the Ontario Cancer Institute in Toronto in 1983. Following post doctoral studies, he became a Member of the Basel Institute for Immunology in Switzerland where he worked for five years, then moved to Vienna Austria where he accepted a post of Visiting Scientist at the Research Institute of Molecular Pathology. In 1990, Keller moved to the United States, working initially at the National Jewish Centre for Immunology and Respiratory Medicine in Denver Colorado and from 1999-2006 as a Professor in the Department of Gene and Cell Medicine at the Mt. Sinai School of Medicine in New York. In 2005, he was appointed as the Director of the Black Family Stem Cell Institute within the Mt. Sinai School of Medicine. As of January 2007, Keller returned to Canada to accept the position of Director of the McEwen Centre for Regenerative Medicine at the University Health Network in Toronto. Dr. Keller is best known for his research in lineage specific differentiation of mouse and human embryonic stem cells.

SESSION 1: PLURIPOTENT STEM CELL EXPANSION, DIFFERENTIATION AND BIOPROCESSING

Chairs: Bill Miller (Northwestern University) and E. Tzanakakis (State University of New York at Buffalo)

Invited Presentation

Stem Cell Bioprocessing: Increasing Efficiency and Integrating Cell Expansion with Differentiation On Microcarriers

Steve K. W. Oh[1], *Allen Chen[1], Jo'an Bardy[1], Lim Yuming[1], Wei Shunhui[2] Ken Chan[1], Tony Goh[1], Zhang Zhiyong[3], Jerry Chan[3] and Shaul Reuveny[1]*

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[2]Singapore Bioimaging Institute
[3]Duke-NUS Medical School and NUH

Our team has developed a microcarrier (MC) platform purposefully designed for a variety of pluripotent and multipotent stem cells: hESC, hiPSC and hfMSC. In this presentation we will demonstrate the application of this platform to simplify the complex process of cell expansion and differentiation in a single bioreactor and increasing the differentiation efficiency of these cells. Two examples will be given, the integration of hiPSC and hESC expansion and their efficient differentiation to neuroprogenitors (NPC) and expansion of hfMSCs and their *in vitro* and *in vivo* osteogenesis.

hiPSC and hESC were propagated in serum free (mTeSR1) stirred MC cultures achieving a high cell yield of 6.1 x 106 cells/ml, while retaining pluripotency. Thereafter in the same stirred reactor, hiPSCs were further differentiated to PSA-NCAM+ NPC neurospheres, achieving another 16.6 fold expansion over a shorter period of 16 days compared to 2D cultures (20 days) without the need for manual picking of neurospheres. This simplified protocol results in a significant 1 log yield improvement over the traditional 2D culture protocol (333 to 371 NPC/pluripotent cell vs. 32 to 53 NPC/pluripotent cell), while maintaining a >80% purity of PSA-NCAM+ NPC. The expanded NPC were shown to be able to differentiate to β-tubulin III+ functional neurons with action potentials.

Human fetal mesenchymal stem cells (hfMSC) were expanded in one liter controlled MC culture bioreactor achieving a density of 6-8 x 105 cells / ml compared to a cell density of 1.2-1.8 x 105 cells / ml in monolayer cultures; a 3 to 4 fold improvement. The cells retained similar high expression levels of MSC surface markers (Stro-1, CD 73, CD90 and CD105) in both systems. Moreover, the efficiency of *in vitro* 2D osteogenic differentiation of hfMSC from MC were similar to the monolayer cultures, 64.6 ± 4 µg calcium/mg of total cell protein and 56.2 ± 4 µg calcium/mg of total cell protein respectively. When hfMSC were seeded on 3D polycaprolactone/tricalcium phosphate (PCL-TCP) scaffolds culture, a higher calcium deposition of 19.2 ± 0.8 mg / mg of total cell DNA was obtained from MC cultures as compared to 12.0 ± 0.9 mg from monolayer cultures. Finally, hfMSC harvested from MC or monolayer cultures mixed with hydroxyapitite and fibrin glue were implanted into the subcutaneous layer of NOD/SCID mice. After 3 months of transplantation, microCT analysis revealed a 1.6 ± 0.2 times more ectopic bone volume in implants from MC cultures. Thus by employing the MC platform a significant increase in both expansion and differentiation was achieved.

Both of these processes have potential for generating huge quantities of anchorage dependent stem cells and their progenitors for preclinical studies.

Engineering 3D Pluripotent Stem Cell Aggregates for Scalable Directed Differentiation

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Pluripotent stem cells (PSCs) can be differentiated to derivatives of all three germ lineages as self-assembled 3D cell aggregates commonly referred to as "embryoid bodies" (EBs). In addition to serving as a unique *in vitro* model system for embryonic development and morphogenesis, suspension cultures of Ebs represent an inherently scalable approach for the biomanufacturing of PSC-derivatives and modular tissue engineered constructs. We have previously demonstrated the ability to entrap microparticles (MPs) composed of synthetic degradable polymers as a means to control the release of

small molecules within the 3D microenvironment of Ebs and thereby induce controlled morphogenesis (1)(2). More recently, we have examined the effects of incorporating ECM-based MPs alone on mouse EB phenotypes or exogenous growth factor delivery from entrapped MPs on EB differentiation (3)(4). Thus, the objective of these studies was to expand upon the utility of biomaterials-based approaches to engineer the biochemical and biophysical properties of 3D PSC environments.

Gelatin and heparin-conjugated gelatin MPs were fabricated using water-in-oil emulsion techniques similar to previously described methods. Heparin was conjugated to gelatin using EDC/NHS chemistry following gluteraldehyde cross-linking of the MPs. MPs were incorporated within Ebs via forced aggregation in PDMS microwells to which mouse ES cells (D3) were first introduced (AggreWells[a], STEMCELL Technologies). After 24-48 hours of formation, the resulting ESC/MP aggregates were subsequently cultured for up to 2 weeks using rotary orbital suspension culture (5). The temporal and spatial patterns of differentiation were assessed by a combination of gene expression, flow cytometry, confocal microscopy and immunohistochemistry.

Delivery of BMP4 or Noggin from gelatin MPs stimulated opposing effects to promote mesoderm or ectoderm morphologies and gene expression (respectively), which were comparable to or exceeded that of soluble treatment controls. The percentage of Brachyury-T+ cells (early mesoderm marker) was increased with BMP4 treatment, and moreso with delivery from MPs than soluble delivery methods. Introduction of heparin-conjugated gelatin and gelatin unloaded MPs alone significantly affected the secreted morphogen profile (BMP4, IGF2, VEGF) of Ebs, suggesting that the presence of the materials sequestered endogenous growth factors which are capable of influencing cell fate decisions. In further support of this notion, divergent gene expression patterns were observed between Ebs containing incorporated heparin-gelatin and gelatin MPs. More specifically, VE-cadherin+ cells were transiently enhanced locally adjacent to heparin-gelatin MPs during the course of EB differentiation. Furthermore, incorporation of magnetic MPs that were physically entrapped during EB formation were used to facilitate subsequent manipulation of the PSC aggregates in suspension culture conditions via externally applied magnetic forces to immobilize and translate populations of magnetic-MP laden Ebs, as well as create multiscale patterns of individual or large numbers of multicellular aggregates.

Overall, these results demonstrate novel applications of engineered micron-scale biomaterials to engineer PSC 3D microenvironments for directed and scalable differentiation. These enabling technologies are anticipated to yield more robust, reproducible and cost-efficient strategies to produce stem cell derivatives and tissue engineered constructs for regenerative medicine and *in vitro* diagnostic applications.

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ROCK-Myosin II Regulation of Embryonic Stem Cell

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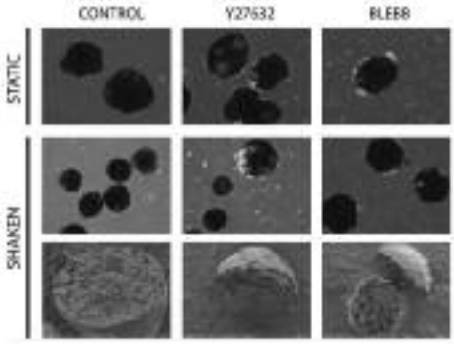
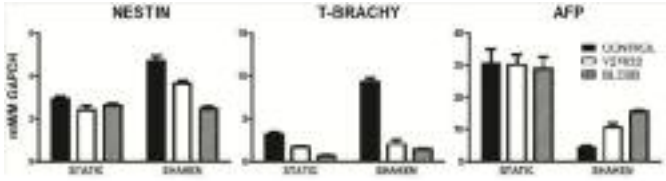
With the advancement of stem cell-based therapies for regenerative medicine and tissue engineering applications, there is an increasing need to understand the means by which to direct differentiation to specific cell phenotypes. Embryonic stem cells (ESCs) are attractive candidates for tissue engineering due to their ability to self-renew, as well as differentiate to the three germ lineages (endoderm, mesoderm, and ectoderm) from which are derived all cells of the body. Germ lineage specification during development is associated with large spatial and morphological rearrangements of cells. Recently, we have shown in a





2D model that applied shear stress affected early ESC differentiation events *in vitro*. It is yet unknown, however, the mechanisms by which the physical microenvironment affects germ specification. Cell processes (e.g. differentiation, migration, and adhesion) are often associated with rearrangements of the cytoskeleton, including actin, which is partially regulated by Rho-ROCK signaling. To better understand the role of the cytoskeleton during differentiation, the objective of this study was to examine the role of the Rho-ROCK-myosin II pathway in ESC germ specification.

Mouse ESCs were seeded on non-adherent dishes to form embryoid bodies (Ebs) in ESC medium without LIF. After 4 days, experimental Ebs were cultured in medium supplemented with Y27632 (3.5 µg/mL) and Blebbistatin (7 µg/mL) to inhibit ROCK and myosin II, respectively. Culture was terminated at Day 7 and samples were assessed for gene expression of ectoderm (NESTIN), mesoderm (T-BRACHY), and

endoderm (AFP) or fixed for imaging. In separate experiments, the treatment conditions were repeated except with culture on a platform shaker system at 40 RPM, which has been shown to apply 1.5 dynes/cm2 to the outer EB surface(1).

Overall integrity of the EB was similar across untreated and treated samples (Figure 1). EB size was heterogeneous in all samples and some agglomeration, which led to EB asymmetry, was observed in static samples. Under both static and shaken conditions, Blebbistatin- (BLEBB) and Y27632-treated samples appear to have larger cavities than control Ebs, as observed by regions of decreased opaqueness in phase images. In addition, when shaken samples were sectioned and imaged with SEM, cavities in the interior of the Ebs were also apparent. Furthermore, the continuous ECAD staining in the outer cells that is consistently observed in control Ebs was disrupted in treated Ebs, indicated a change in cell-cell interactions. Thus, while perturbations of the ROCK-myosin II pathway did not dramatically alter general EB morphology, distinct changes occurred in the spatial organization and cell-cell interactions of cells.

Differentiation events were modulated by both applied shear and drug treatment (Figure 2). Under static conditions, drugs had no effect ($p>0.1$) in the expression of ectoderm and endoderm. With the application of shear stress, expression of NES and AFP increased ($p<0.05$) and decreased ($p<0.01$), respectively. Additional treatment with BLEBB and Y27632, however, mitigated the shear-induced response. Mesodermal differentiation, on the other hand, was consistently responsive to drug treatments under both static and shaken conditions, with marked decreases in T-BRACHY expression ($p<0.001$). Thus, the ROCK-myosin II pathway is important for mesodermal differentiation, as well as for all germ lineage specification within a dynamic mechanical environment.

Actin stress fibers at focal adhesion complexes create a counter balance to forces imposed by the microenvironment (2). Through the use of protein inhibitors, this study perturbed the ROCK-myosin II pathway, in part responsible for myosin-actin dynamics. Resulting changes in force distribution throughout the cell affected gene expression within the nucleus, leading to altered differentiation patterns.

Studies by many others have shown that Rho-ROCK signaling is important in mechanotransduction, including regulation of MSC differentiation (3) and shear stress activation of sterol regulators in endothelial cells (4). These cells of the mesodermal lineage, which also include smooth muscle cells, skeletal muscle cells, and fibroblasts, are actively mechanoresponsive to physical forces in situ. This study indicates, however, that either the ability to transfer external forces to the nucleus or the ability to support intracellular tension may be required for differentiation towards mechanoresponsive phenotypes. Thus, manipulation of the cytoskeleton may be an effective strategy for directed differentiation towards target phenotypes.

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Wnt/β-Catenin Signaling Promotes Differentiation, Not Self-Renewal, of Human Embryonic Stem Cells and Is Repressed by Oct4

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Signal transduction pathways play diverse, context-dependent roles in vertebrate development. In studies of human embryonic stem cells (hESCs), conflicting reports claim Wnt/β-catenin signaling promotes either self-renewal or differentiation. We use a sensitive reporter to establish that Wnt/β-catenin signaling is not active during hESC self-renewal. Inhibiting this pathway over multiple passages has no detrimental effect on hESC maintenance, while activating signaling results in loss of self-renewal and induction of mesoderm lineage genes. Following exposure to pathway agonists, hESCs exhibit a delay in activation of β-catenin signaling, which led us to postulate that Wnt/β-catenin signaling is actively repressed during self-renewal. In support of this hypothesis we demonstrate that OCT4 represses β-catenin signaling during self-renewal and that targeted knockdown of OCT4 activates β-catenin signaling in hESCs. Using a fluorescent reporter of β-catenin signaling in live hESCs we observe that the reporter is activated in a very heterogeneous manner in response to stimulation with Wnt ligand. Sorting cells based on their fluorescence reveals that hESCs with elevated β-catenin signaling express higher levels of differentiation markers. Together these data support a dominant role for Wnt/β-catenin signaling in the differentiation rather than self-renewal of hESCs.

SESSION 2: ADULT STEM CELL ISOLATION, EXPANSION, DIFFERENTIATION AND BIOPROCESSING

Chairs: Jon Rowley (Lonza) and Johnna Temenoff (Georgia Institute of Technology)

Invited Presentation

NOTCH-Mediated Regulation of Hematopoiesis: Basic and Clinical Implications

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The factors governing hematopoietic stem cells (HSC) are not well understood, but are thought to result from interplay between soluble factors such as cytokines, and intercellular interactions, including those with marrow stroma. These latter interactions involve a number of evolutionary conserved pathways known to govern cell fate decisions in a wide variety of developing systems, such as the Notch pathway. Studies of hematopoiesis have revealed a role for Notch signaling in reg-

ulating early lymphoid development, specifically in regulating T vs. B cell-fate decisions. Although the role of Notch signaling in regulating hematopoietic stem cell fate decisions remains controversial, our recent studies have provided evidence for an *in vivo* role of Notch2 in regulating the tempo of HSC growth and differentiation during marrow regeneration. *in vitro* studies, however, have shown profound effects of enforced expression of the constitutively active intracellular domain of Notch1 or activation of endogenous Notch receptors using an immobilized, engineered form of the Notch l igand Delta1 (Delta1ext-IgG) in inhibiting myeloid differentiation, promoting early T cell differentiation and, enhancing the cytokine dependent generation of short-term marrow repopulating cells.

In preclinical studies, culture of human cord blood precursor cells with Delta1ext-IgG led to an approximate 15-fold increase in the number of NOD/SCID repopulating cells and provided more rapid engraftment compared to non-cultured cells. In an ongoing Phase I clinical trial, patients undergoing a myeloablative CBT are receiving one non-manipulated CB unit along with a second CB unit that has undergone Notch-mediated *ex vivo* expansion. In the first eleven patients enrolled on this trial, an average of 5.9 x 10 6 CD34+ cells/kg resulting from an average 164-fold expansion of isolated CD34+ cells were co-infused with an average 2.3 x105 cells/kg from a second non-manipulated unit. These cells were safely infused and led to a significant reduction in the time to neutrophil engraftment. A median time to absolute neutrophil count (ANC) ≥ 500 per µl of 14.5 days was observed in those receiving the expanded unit as compared to 25 days in a concurrent cohort of 29 patients receiving dCBT with the same conditioning and post-transplant immunosuppressive regimen (p=0.0002). We also demonstrated a statistically significant improvement in median time to achieve an ANC > 100 cells per µl (8 versus 19 days, p=0.0015), a potentially important metric based on its association with improved survival after allogeneic stem cell transplant. Median time to platelet engraftment for platelets > 20,000 and 50,000 occurred at 32 (range 19-56) and 45 (range 22-68) days respectively. Whereas peripheral blood leukocytes at 7 days were derived from the expanded units in all patients, at the time of achieving early engraftment with an ANC ≥ 500 per µl only half of the patients demonstrated predominant engraftment with the expanded CB unit while the other half had engraftment derived from the non-manipulated unit suggesting that the expanded unit may have facilitated engraftment by the non-manipulated unit. Recent studies indicate that administration of higher CD34+ doses can further reduce the time to neutrophil engraftment, and the possibility of using a cryopreserved "off-the-shelf" expanded cell product to provide a more cost-effective approach. Overall, these studies show promise for shortening time to neutrophil and platelet engraftment in patients undergoing cord blood stem cell transplantation.

Bioengineering Approaches for the Optimization of the Ex-Vivo Expansion of Hematopoietic Stem/Progenitor Cells for Cell Therapy

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The major limitation to a wider use of umbilical cord blood (UCB) in hematopoietic stem/progenitor (HSC) cell therapy is the low cell dose available. However, the major focus in HSC expansion is still the rational delineation of optimal culture conditions to amplify HSC numbers, which have a critical impact on the final quality of the expanded graft and on the cost effectiveness of the expansion process.

In this study, UCB CD34+-enriched cells were co-cultured with mesenchymal stem cell (MSC)-derived feeder layers using a cytokine-supplemented serum-free medium. Using a stem cell engineering approach, important parameters affecting ex-vivo expansion performance of UCB HSC were systematically studied and optimized, while reducing culture costs by 50-65% compared to previously established protocols: i) A design of experiments strategy was applied to the optimization of serum-free media formulations and cytokine cocktails, resulting in an increased cell productivity (20-fold increase in CD34+ cells after 7 days); ii) The impact of the initial CD34+ cell content was studied showing that high (>90% CD34+ cells) initial progenitor content was not mandatory to successfully expand HSC, since cell populations with moderate levels of enrichment (Low: 24±1.8% and Medium: 46±2.6%) readily increased CD34 expression, generating higher stem/progenitor cell yields. Iii) In addition, the effect of oxygen tension in HSC-MSC co-cultures was investigated: physiological O2 levels (5-10%) were found to be beneficial for an efficient expansion of UCB HSC while cell metabolic patterns were consistent with hypoxic adaptation, being shifted to aerobic glycolysis. Overall, the results presented herein give important insights to better understand the cellular determinants underlying ex-vivo expansion of HSC, providing the basis for the establishment of efficient and controlled culture systems for the generation of clinically significant cell numbers in the settings of BM transplantation using UCB expanded cells.

Larger Scale Expansion of Human Mesenchymal Stem Cells On Microcarriers

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[2]Birmingham University, Birmingham, UK

The effects on human mesenchymal stem cell growth of choosing either of two spinner flask impeller geometries, two microcarrier concentrations and two cell concentrations (seeding densities) were investigated. The minimum agitation speed for microcarrier suspension, NJS, was measured for each impeller and when agitation was employed, that speed was chosen for the actual growth runs. Initially, without growth, it was shown microcarriers were not damaged when agitated at that speed. With culture, the maximum cell density was achieved after 8 to 10 days with up to a 20 fold expansion in terms of cells per microcarrier. An increase in microcarrier concentration or seeding density generally had a deleterious or neutral effect, as previously observed for human fibroblast cultures. The choice of impeller was significant, as was incorporation of a 1 day delay before agitation to allow initial attachment of cells. The best conditions for cell expan-

sion on the microcarriers in the flasks were 3000 microcarriers mL-1 (ca. 1 g dry weight L-1), a seeding density of 5 cells per microcarrier with a 1 day delay before agitation began at NJS (30 rpm), using a horizontally suspended flea impeller with an added vertical paddle. These findings were interpreted using Kolmogorov's theory of isotropic turbulence. In all the culture conditions tilized, the cell quality was assessed by microscopic observation, the currently used technique for T-flask cultures. No observable difference in quality could be seen.

Isolation of Specific Progenitor Cells with Label-Free DEP Sorting

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Generation of differentiated neurons and astrocytes from neural stem/progenitor cells (NSPCs) of the developing cerebral cortex occurs via the production of progenitors biased to either neuronal or astrocytic fates. Despite developmental studies documenting these progenitors, a precise description of their discriminating cellular characteristics is lacking, partly due to limitations in available markers for these progenitors. We previously found a label free technique for characterizing cells, dielectrophoresis (DEP), distinguishes both human and mouse neuronal progenitors from their astrocytic counterparts. Progenitor cell dielectric properties measured by DEP shift as the cells lose neurogenic potential over time in culture and a specific measure of the membrane properties of the cells, membrane capacitance, is clearly linked to cell fate potential.

DEP has been used successfully in other contexts to sort distinct cell populations. Our studies have shown exposure to DEP forces necessary for cell sorting does not harm human or mouse NSPCs, clearing the way for possible DEP-based sorting of these cells. We therefore used DEP to isolate cells from NSPCs containing both neuronal and astrocytic progenitors and found that astrocytic progenitors were isolated at lower DEP frequencies while neuronal progenitors were collected at higher frequencies. These results show that dielectric properties defining specific progenitor cell populations can be used for their isolation. Specific dielectric properties can be added to the list of characteristics that distinguish neuronal from astrocytic progenitors and isolation of these progenitors by DEP can provide purified populations of cells for further analysis and determination of distinct cellular properties.

Keynote Presentation

Feedback Control of Endogenous Signaling to Guide Stem Cell Fate

Peter Zandstra
Research in the Zandstra Laboratory is focused on the generation of functional tissue from adult and pluripotent stem cells. His groups' quantitative, bioengineering-based approach strives to gain new insight into the fundamental mechanisms that control stem cell fate and to develop robust technologies for the use of stem cells and their derivatives to treat disease. Specific areas of research focus include blood stem cell expansion and the generation of cardiac tissue and endoderm progenitors from pluripotent stem cells. Dr. Zandstra is a Professor in the Institute of Biomaterials and Biomedical Engineering, the Department of Chemical Engineering and Applied Chemistry, and the Donnelly Centre at the University of Toronto. He is also a member of the McEwen Centre for Regenerative Medicine and the Heart and Stroke/Richard Lewar Centre of Excellence. He currently acts as Chief Scientific Officer for the Centre for the Commercialization of Regenerative Medicine (www.CCRM.ca). Dr Zandstra's accomplishments have been recognized by a number of awards and accolades including a Guggenheim Fellowship and the McLean Award. Dr. Zandstra's strong commitment to training the next generation of researchers is evidenced by his role as the Director of the undergraduate Bioengineering Program.

SESSION 3: ENGINEERING ENVIRONMENTS TO CONTROL CELL FATE

Chairs: Andre Levchenko (Johns Hopkins University) and Joel Voldman (Massachusetts Institute of Technology)

Invited Presentation

Harnessing Endogenous Signals In the Stem Cell Microenvironment

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The extracellular matrix (ECM) plays a critical role in stem cell behaviors ranging from cell adhesion to lineage-specific differentiation. A large number of studies to date have focused on understanding the diverse role of the ECM in regulating stem cell behavior. However, the influence of the ECM on stem cell behavior is often difficult to study in traditional cell culture, as the extracellular environment in cell culture is often complex and poorly defined. We have hypothesized that chemically well-defined cell culture environments can be used to study the influence of ECM-mimicking (or "bioinspired") molecules on stem cell behavior. A particular focus has been on developing and using chemically well-defined platforms that present diverse molecules, alone or in combination. Specifically, we have used bio-inert self-assembled monolayers (SAMs) or poly(ethylene glycol) (PEG) lydrogels, chemically modified with bioinspired ligands. Bioinspired ligands have included growth factor sequestering ligands, proteoglycan sequestering ligands, and cell adhesion ligands. Our results demonstrate that peptide ligands can significantly influence diverse stem cell behaviors, including adhesion, proliferation, migration, phenotypic transformation, and lineage-specific differentiation. In addition, the context in which specific ligands are presented to stem cells critically influences cell behavioral responses. Importantly, the use of chemically well-defined biomaterials in these studies helps to eliminate the confounding factor of random, non-specific biomolecule adsorption, and enables identification of specific bioinspired ligands as key mediators of stem cell behavior. Recent studies in this area have focused on harnessing endogenous signals to amplify specific stem cell behaviors, including expansion and lineage-specific differentiation. Ongoing studies in this

area are discovering key mechanisms for stem cell regulation and understanding dynamic responses of stem cells to their physical and biochemical microenvironment.

Diblock Copolymer Foams with Adhesive Nano-Domains Promote Stem Cell Differentiation

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Focal adhesions are important transducers of signals from the extracellular matrix (ECM) to the cell and vice versa. Most biomaterials are coated with an ECM ligand uniformly to promote adhesion, but that does not match heterogeneous adhesive site distribution in native ECM. Mixed copolymer vesicles undergo interface-confined phase separation; by using diblock copolymer mixtures of non-adhesive and adhesive components, i.e. polyethylene oxide (PEO)-polystyrene (PS) and polyacrylic acid (PAA)-PS, respectively, we determined if foam structures made in a high internal phase emulsion would have surfaces composed of adhesive and non-adhesive domains just as with ECM. Bulk copolymer incorporation scaled with input composition but neither foam morphology nor surface roughness dramatically changed as a result. However, surface phase separation did occur between PAA-PS and PEO-PS; 0.1 μm2 nano-domains spaced ~0.5 μm apart were found when either copolymer fraction was ≤25%. That size and spacing is similar to the heterogeneous adhesivity of native ECM. When incubated with an ECM ligand, protein attached to PAA-PS and produced nano-domains with a distribution that also mimicked native ECM. Two human mesenchymal stem cell sources cultured on the foams were adherent on and express the most robust vinculin-containing adhesions on 25% PAA foams. qPCR microarray data indicates that these two mesenchymal stem cell sources undergo both nano-domain-dependent and –independent differentiation depending on the lineage to which the cell commits. Thus interface-confined phase separation in copolymer foam mixtures can create adhesive nano-domains mimicking native ECM, induce stem cells to differentiate, and should be considered in future regenerative strategies.

Micromolded Hydrogel Substrates of Tunable Stiffness

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Cornell University, Ithaca, NY

Recent data from our lab shows that the mechanical properties of the extracellular matrix can influence cell-cell connectivity and drive the formation of vascular networks. Data from others has indicated that topographical features within the microenvironment can be used to direct cell movements. So while both substrate topography and substrate mechanical properties are known to influence cell behavior, little is known about how they act in concert. Here, a method is presented to introduce topographical features into polyacrylamide hydrogel substrates that span a wide range of physiological Young's moduli (E). Gel swelling plays a significant role in the fidelity of protruding micromolded features, with the most efficient pattern transfer occurring at a crosslinking concentration equal to or greater than ≈5%. In contrast, swelling does not influence the spacing fidelity of microcontact printed islands of collagen on 2D polyacrylamide substrates. Cells cultured on micromolded polyacrylamide substrates exhibit contact guidance along ridges patterned for all Young's moduli tested. These methods will be useful for presenting cells well-controlled mechanical and topographical cues.

Generating Functional T Cells From Stem Cells: On-Demand and Renewable Cell Immunotherapy

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[2]Department of Biomedical Engineering, The University of Texas at Austin,

[3]Dell Pediatric Research Institute, The University of Texas at Austin

Immunotherapy by adoptive transfer with autologous T cells has shown promise for the treatment of many cancers and post-transplant lymphoproliferative diseases. During the process of adoptive transfer, T cells are isolated from a patient's peripheral blood, expanded, selected, and trained for antigen specificity before being returned to the patient. This method is severely constrained by inefficient isolation, problems with primary cell culture, and limited availability of donor cells. Multipotency of stem cells makes them attractive alternative sources from which functional T cells can be generated *in vitro*.

Two insoluble signals presented by thymic stromal and antigen presenting cells are essential for T cell development: Notch receptor signaling induced by Delta-like ligands (DLL) and T cell receptor (TcR) signaling induced by Major Histocompatibility Complexes (MHCs). Most T cell differentiation efforts to date focus on mimicking these signals in 2D co-culture systems with bone-marrow derived stromal cells expressing retrovirally transfected Notch ligands (OP9-DL1 cells). Thus, there is need for a system that supports T cell differentiation in a controlled, stroma-free approach suitable for therapeutic and clinical purposes. Previous studies show that microbeads with surface-immobilized Notch ligands can efficiently direct hematopoietic progenitor cells to the early T cell lineage. In addition, we have demonstrated that by using a combination of surface-immobilized Notch ligand-mediated signaling and soluble LCMV peptide-loaded MHC tetramer-mediated TcR signaling, mouse embryonic stem (ES) cells can be directed to the T cell pathway and differentiate into functional, LCMV-specific CD8+ T cells.

Recently, we demonstrated that human CD34+CD38- cord-blood (CB) derived hematopoietic stem cells (HSCs) can differentiate into functional, antigen-specific T cells in a stroma-free system. Plate-bound Notch ligands induced T lineage commitment and gave rise to early and CD4+CD8+ double positive (DP) T cells. Subsequent addition of CMV-pp65 HLA tetramers or peptide resulted in the generation of CMV-specific CD8+ T cells. CD8+CMV+ T cells co-cultured with CMV peptide-loaded target cells in a CTL assay secreted cytokines and up-regulated surface expression of a cytotoxic marker, indicating functionality. To investigate a possible mechanism for *in vitro* positive selection with MHC/HLA tetramers, we performed TCR Vbeta repertoire analysis. CMV-specific CD8+ T were found to be polyclonal, with little variation among CB samples, suggest-

ing that TCR editing could be involved in positive selection.

We are now translating the Notch-DLL and MHC-TcR signaling pathways into 2D and 3D biomimetic soft material-based systems that will more closely mimic the thymic microenvironment. We have fabricated flat hydrogel surfaces or porous hydrogel scaffolds that can present immobilized DLL or MHC molecules to seeded cells. A polyacrylamide-based gel of different material stiffness was modified to immobilize DLL at varying, controllable densities. Mouse hematopoietic stem cells seeded on these gels of varying material stiffness and different DLL-densities differentiated into early T cells. Inverse opal porous scaffold from poly(ethylene glycol) functionalized with binding moieties such as protein A or streptavidin were fabricated. We are able to control and vary the density of Notch ligands and MHC molecules on these scaffolds, as well as induce Notch signaling of varying strengths in cells seeded within the scaffolds, as indicated by expression of a downstream Notch gene. This system allows for the controlled presentation of essential T cell differentiation ligands in a stromal cell-free system. We hypothesize that the generated 2D and 3D soft microenvironments will induce Notch-DLL and MHC-TcR signaling in a more biomimetic way and provide an engineered system in which complete differentiation of stem cells into functional T cells can be performed.

SESSION 4: STEM CELL MODELS OF DEVELOPMENT AND DISEASE

Chairs: Tabassum Ahsan (Tulane University) and Adam Engler (University of California, San Diego)

Invited Presentation

Stem Cell Behavior As Controlled by Intrinsic Mechanisms

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Stem cell hydrogel is controlled by intrinsic mechanisms, such as transcription factors, and by external signals from the local microenvironment or niche. Using adult epidermis as an experimental model, my lab is investigating the interplay between specific intrinsic and extrinsic signals in regulating stem cell fate. We are currently using a range of *in vitro* approaches, including the generation of micro-patterned substrates that capture single stem cells or facilitate the assembly of a micro-epidermis comprising 5-10 cells. Our studies highlight the importance of the Wnt, Notch, EGF receptor and integrin pathways and help us to understand how oncogenic changes in cells of multi-layered epithelia impact on the underlying tumour stroma.

Dystrophin Expression Restoration by Human Artificial Chromosome In Cardiomyocytes Derived From DMD Patient hips Cells

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Human artificial chromosomes (HACs) are powerful tools for gene therapy approaches since they are stably maintained in human cells without integration into the host genome and they have the capacity to carry large genomic loci. Human induced pluripotent stem hips cells have great potential for cell therapy, as such cells can be generated from the patient's own tissues and have the capacity to differentiate into all cell types of the human body. Oshimura and colleagues recently demonstrated the complete correction of hips cells derived from a Duchenne Muscular Dystrophy (DMD) patient, using a HAC carrying the full-length genomic dystrophin sequence (DYS-HAC), including also the associated regulatory elements(1). The combination of hips cells technology with an HAC vector containing the normal version of a defective dystrophin gene have pioneered an innovative and promising therapeutic approach for the treatment of DMD. However, in order to develop and screening for new therapeutic approaches, it is necessary to differentiate the genetically-corrected hips cells into cardiomyocytes with mature phenotype, in particular, with a proper expression and subcellular localization of the various dystrophin isoforms and all splicing variants. In this work, we obtained human cardiomyocytes (hCMs) from both healthy, DMD and HAC-corrected hiPSc. This hCMs were used as a model for studying the ability of the DYS-HAC to restore different dystrophin isoforms during cardiogenesis, compared to healthy and DMD patient-derived hips cells. Embryoid bodies (Ebs) were generated from hips cells and differentiated in suspension culture using a defined temporal sequence of cytokines as previously reported by Keller and colleagues (2). A 40% of spontaneously contracting Ebs were obtained. Cells from contracting Ebs were then cultured on substrates with physiological stiffness for additional 6 days. An adhesion-dependent maturation of hCMs was observed independently from suspension culture time (for Ebs older than 20 days). In particular, in these conditions hCMs were shown to develop a defined sarcomeric architecture of cTnT and α-actinin, further to a shortening in calcium transients (down to 0.3 sec). The expression of cardiac-specific transcription factors such as GATA4 and NKX2.5, of the gap-junction protein connexin 43 and of the atrial isoform of myosin light chain was revealed by immunefluorescence and RT-PCR analyses. The DYS-HAC was shown to drive the expression of dystrophin sequences originally deleted in the DMD patient. Furthermore, dystrophin expression was correctly restored at membrane level in hCMs. Multiple dystrophin isoforms were shown to be expressed during the differentiation process (long muscular isoforms Dp427m and Dp427l and short isoforms such as Dp140 and Dp71). The expression of a dystrophin isoform at nuclear level was also observed, according to the finding that Dp71 isoform can display a nuclear and nuclear envelope localization during myogenesis (3). Finally, our results demonstrate how the DYS-HAC allows a complete genetic correction of hCMs derived from DMD patient-specific hips cells. The obtained hCMs can represent a valuable cell source for modeling DMD-associated cardiomyopathy and, more in perspective, for the development of a therapeutic strategy for DMD, effective not only on skeletal muscle tissues but also at cardiac level.

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Bioengineering Embryonic Stem Cell-Breast Cancer Cell Interactions to Understand Metastasis

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Breast cancer is the most prevalent disease among women worldwide. Metastasis is the leading cause of cancer death. The interaction between breast cancer cells and their microenvironments plays a crucial role in cancer metastasis. Invasive breast cancer cells have been linked to embryonic stem cells (ESCs) due to certain similar gene signatures and the convergence of tumorigenic and embryonic pathways. However, embryonic signaling molecules are misexpressed in invasive cancer cells and critical regulators of these signaling pathways are missing in a tumor microenvironment. Since embryonic signaling pathways are tightly controlled in ESCs, bioengineered ESC microenvironments have great potential to supply critical signaling molecules and reprogram the abnormal embryonic signaling pathway in tumors. In this study, we have bioengineered embryonic microenvironments using alginate lydrogels and utilized *in vitro* dynamic embryonic microenvironments to understand malignant growth and migration of metastatic breast cancer cells. Our results have shown that mouse ESCs could self-assemble and grow in tunable alginate hydrogel microstrands, microbeads or nanofibers while maintaining their self-renewal ability and pluripotency. In particular, we have demonstrated that bioengineered embryonic microenvironments could inhibit the proliferation and migration of highly invasive breast cancer cells, indicating the potential to reprogram metastatic cancer cells to a less malignant phenotype. Using this bioengineered ESC-breast cancer model system, we further characterized the metastasis phenotype of breast cancer cells and explored the factors to restrict breast cancer metastasis. This bioengineering approach provides a unique opportunity for better understanding and effectively restricting metastasis.

Pluripotent Stem Cell Derived Models of Human Trophoblast Differentiation *in vivo*

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Formation of the trophectoderm (TE) and the inner cell mass (ICM) are the first lineage decisions taken by the developing embryo. Upon embryo implantation, TE cells give rise to villous cytotrophoblasts (vCTBs), which further differentiate into syncytiotrophoblasts (STBs), column cytotrophoblasts (cCTBs) and invasive cytotrophoblasts (iCTBs).

The early signaling events that lead to TE formation and its subsequent differentiation to STBs and iCTBs remain poorly characterized. Studies in mice have provided great insight into the regulation of TE formation and subsequent differentiation. Nonetheless, the applicability of mouse models to human TE formation is yet to be confirmed. Primary cells derived from human placentas have been extensively used to study trophoblast differentiation. However, these cells are already lineage committed and cannot be used to explore the molecular mechanisms underlying lineage commitment. Primary vCTBs also exhibit molecular differences across first, second and third trimesters; primary vCTBs from the first trimester spontaneously form iCTBs while those from the third trimester have been reported from STBs *in vitro*. Alternatively, trophoblast choriocarcinoma cell lines, such as JUG3, JAR, AC1M-88 and AC1M-32 have been used as model systems to investigate trophoblast biology. While convenient, these cell lines display variability in their molecular characteristics, and also differ from primary cells. Moreover, results obtained from these cell lines may not be applicable to primary cells due to genetic alterations present in these cell lines.

More recently, hESCs have been used as a source to obtain TE derivatives. Trophoblasts have been obtained through treatment with Bone Morphogenetic Protein (BMP) or embryoid body (EB) formation. However, variability in response towards BMP treatment has been observed among various hESC cell-lines. Notably, upregulation of mesoderm genes, extraembryonic endoderm genes as well as spatial variations within the differentiating colony have been reported. The EB method does not allow for a mechanistic understanding of the early steps leading to TE commitment due to inherent heterogeneity in the EB microenvironment. Moreover, trophoblasts obtained from both BMP-treated hESCs and Ebs do not upregulate CDX2, a key marker of trophoblast.

We have shown that vCTBs can be obtained through blocking of the Activin/Nodal pathway. Previous studies have shown that Smad1 signaling plays an important role in trophoblast differentiation; indeed, simultaneous inhibition of Activin/Nodal and BMP pathways leads to neural differentiation in hESCs. Our data shows that phosphorylated Smad1 levels are maintained during differentiation to vCTBs and supports the hypothesis that BMP signaling acts as a competence factor in trophoblast differentiation. We have also carried out a comprehensive proteomic characterization of vCTBs. Our results confirm the expression of several protein markers of vCTBs and also provide insight into the molecular pathways that may be active in these cells.

The vCTBs obtained from hESCs are multipotent and can be further differentiated to multinucleate STBs, or alternatively cCTBs and iCTBs, through manipulation of Activin/Nodal signaling. We have developed protocols to isolate pure populations of these differentiated cell types. Gene expression analysis, immunofluorescence and morphological characterization confirm the phenotype of the differentiated cells. Thus, taken together, our results show that pluripotent cells can be used as models of trophoblast differentiation and placental development.

Mesoderm Differentiation and Patterning in Human Embryonic Stem Cells

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Ortmann, Lily Cho, Kathy Niakan, Sasha Mendjan, Andreia Bernardo and Sanjay Sinha
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The discovery of human embryonic stem cells (hESCs) generated great enthusiasm for their potential use in regenerative medicine. These expectations were based on their pluripotency, a property shared with mouse embryonic stem cells (mESCs), which were previously shown to be capable of forming all body tissues in chimaeric mice. The extensive use of mESCs to generate fully normal chimaeric mice, without causing any birth defects or other proliferative anomalies, indicates that developmental properties of mESCs closely resemble those of the inner cell mass (ICM), from which they were derived. However, in the years since their discovery, hESCs have been shown to differ significantly in developmental properties from mESCs or the ICM itself. Indeed, hESCs appear instead to closely resemble pluripotent stem cells that have been derived from the late epiblast layer of post-implantation mouse embryos (EpiSCs). EpiSCs respond similarly to hESCs when exposed to growth factor conditions that either maintain pluripotency or induce their differentiation into neuroectoderm, mesoderm or endoderm. Accordingly, the full capability of hESCs and EpiSCs for normal tissue development and proliferative stability remains to be demonstrated, as they are dissimilar to mESCs and EpiSCs do not form chimaeras efficiently. We have undertaken studies on the responses of hESCs and EpiSCs to conditions that induce their differentiation into mesodermal lineages. We find that hESCs and EpiSCs respond to combined bone morphogenetic protein (BMP)-4 and fibroblast growth factor (FGF)-2 treatment by differentiating into a mesodermal phenotype closely resembling lateral mesoderm of the gastrulating embryo. Moreover, these mesoderm cells can be further induced to form smooth muscle and cardiomyocytes that express marker genes appropriate to the endogenous lateral mesoderm-derived tissues. Our findings thus indicate that hESCs and EpiSCs recapitulate known fates for lateral mesoderm during mouse gastrulation. This supports the characterisation of hESCs and EpiSCs as valid models for understanding developmental events ranging from pluripotency through tissue patterning. (Supported by the UK Medical Research Council, British Heart Foundation, the Wellcome Trust and the March of Dimes Foundation.)

SESSION 5: CELLULAR REPROGRAMMING

Chairs: Balaji Rao (North Carolina State University) and David Schaffer (University of California, Berkeley)

Invited Presentation

Human Induced Pluripotent Stem Cells

Joseph C. Wu
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The successful derivation of human induced pluripotent stem cells (hiPSCs) by de-differentiation of somatic cells offers significant potential to overcome obstacles in the field of cardiovascular disease. hiPSC derivatives offer incredible potential for new disease models and regenerative medicine therapies. However, many questions remain prior to clinical translation in the future. This talk will highlight on anticipated clinical hurdles of pluripotent stem cell therapy, focusing on issues such as donor cell source, differentiation, immunogenicity, and tumorigenicty.

Single-Cell Dynamics of Cell Lineage Commitment

Tyler Gibson *and Charles A. Gersbach*
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Regenerative medicine offers significant promise to repair damaged tissues and treat degenerative disease. The ability to control cellular differentiation is essential for cell-based therapies. Genetic reprogramming is a powerful tool to direct cellular differentiation through the delivery of transcription factors that regulate gene expression (1). The mechanisms of cell lineage commitment are not fully understood, and further study of this process can improve the development of regenerative therapies.

Several studies in the past decade have shown a large degree of variability in gene expression among genetically identical cells, which has raised the question of whether individual cells behave in a manner distinct from the population average. Conventional methods that measure mean protein or gene expression values of cell populations do not provide information about individual cells. Previous studies have suggested that cell lineage commitment is a digital, switch-like response into a distinct phenotypic state. In this case, measurements at single-cell resolution show multimodal distributions corresponding with distinct cell states. Conversely, other studies show cellular changes as analog, graded responses to differentiation stimuli. Individual cells exhibiting this type of response show phenotypes proportional to the magnitude of the stimulus. As a result, the whole cell population shows a graded transition between states (2). The dynamics of switch-like and graded cell responses is determined by the underlying gene regulatory network, with inhibitory feedback loops and self-activating positive feedback commonly seen in networks displaying bistability (3). Therefore, single-cell studies offer another perspective on how these regulatory networks determine cell behavior.

Our objective is to study cell lineage commitment at the single-cell level to gain a better understanding of the dynamics during induced differentiation and the regulatory network structures that guide this process. The master transcription factor MyoD has been used to reprogram fibroblasts into myoblast-like phenotypes by a well-characterized regulatory network. We have developed high-throughput methodologies to enable a population-wide analysis of individual cellular behavior and analyzed single-cell responses to MyoD overexpression. Our preliminary results show that activation of a downstream reporter, as an indicator of lineage commitment, is proportional to the level of forced MyoD expression, suggesting a graded response to this master regulator of differentiation.

An inducible lentiviral system for gene delivery was constructed from an improved single-vector Tet-On system (4). MyoD was placed under control of the tetracycline-responsive element and co-translationally expressed using a T2A “skipping” peptide linking it to the fluorescent protein DsRed-Express2. This construct enabled tracking of expression levels of the delivered transcription factor. The lentiviral vector also included a puromycin resistance gene, which enabled selection for cells that contain the

lentivirally-delivered transgene. The addition of doxycycline to the cell culture media induced MyoD expression to drive reprogramming. Quantitative reverse transcriptase PCR (qRT-PCR) and Western blotting confirmed the dose-dependent expression of MyoD in transduced NIH3T3 mouse embryonic fibroblasts.

Downstream markers of myogenic differentiation include myogenin, troponin T, desmin, and myosin light chain 1. The expression of these markers in the bulk cell population was measured by qRT-PCR, which demonstrated increased expression of all these genes that was proportional to the level of MyoD induction. Western blotting corroborated these results by also showing a dose-dependent increase in myogenin protein levels.

To study reprogramming at the single-cell level, 3T3 mouse fibroblasts were genetically modified to include an eGFP-based reporter under control of the myogenin promoter. This reporter cell population was transduced with the MyoD-containing lentivirus, and expression was induced at various levels. Measurements using flow cytometry gave MyoD expression levels and myogenin promoter activation as red and green fluorescence intensities, respectively. The results showed that varying doxycycline changed both the red and green fluorescence levels in a dose-dependent manner and the myogenin promoter response shifted uniformly relative to the magnitude of the MyoD transgene expression.

These results suggest that MyoD-induced reprogramming does not necessarily involve transitioning across a digital on/off "switch", but can stimulate graded responses to increasing MyoD levels. This is in contrast to previous reports that suggest a cell must cross a threshold or critical "dose" of stimulus to commit to a new lineage, which would be consistent with a bimodal population of cells exhibiting differing myogenin expression levels. Ongoing work in our lab includes analyzing MyoD expression levels during natural differentiation *in vitro* to determine how physiological MyoD induction compares to forced expression. We are also studying the mechanisms by which robust behavior of cell populations emerges from stochastic gene expression in single cells during cell differentiation, and how these mechanisms are altered during transcription factor-driven direct reprogramming.

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3D Culture of Adherent Mesenchymal Cells for Improved Performance In Cell Therapy

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Adherent MSC-like stromal cells (ASC) are favorable candidates for cell therapy, primarily due to their availability and potent secretome. We have shown that placenta derived ASCs induce secretome dependent pro-angiogenic and anti-inflammatory responses in-vitro and in-vivo both in animals and in humans. 12-month data from two Phase I clinical trials have shown that Pluristem's placenta derived ASC product candidate, PLX-PAD, is safe and potentially efficacious in treating patients suffering from Critical Limb Ischemia (CLI). Based on this data it can be said that placental ASCs grown in 3D environment are a promising cell source for treatment of ischemic and immune related diseases. However, production of large numbers of cells for the clinic using 2D culture based platforms has limitations in terms of scalability and process control. To meet the production demand (trillions) of cells that are needed for clinical use we culture ASCs in bioreactors on non-woven fiber carriers. This allows production of large numbers of cells while enabling control over cell culture parameters, and confers to the cells' advantageous biological attributes. To examine the biological effects of our 3D expansion method, placental derived ASC were cultured on 2D tissue culture polystyrene (TCPS), and 3D non-woven fiber scaffolds in spinner flasks and in packed-bed bioreactors. The cells from each growth system were analyzed for ECM deposition, cytoskeleton structure and angiogenic/inflammatory clinically relevant genes and proteins. We found that the biology of ASCs cultured in this 3D method was significantly different from ASCs grown in 2D culture. In 3D culture, ASCs deposit large quantities of collagen and fibronectin, creating an ECM based tissue-like niche. In addition, the cytoskeleton structure observed in 3D differs from that observed in 2D culture where ASCs contain many actin stress fibers. Culture of ASC in the 3D scaffold results in multi-fold up-regulation of angiogenic related genes and >2 fold increase in the secretion of pro-angiogenic proteins as compared with ASCs cultured on 2D TCPS. From the cell expansion perspective, the number of harvested ASCs from one 3D packed-bed (1.5L) bioreactor is equivalent to ASC harvested from 100 2D 10-stack 'cell factory' (Nunc). Taken together, these data demonstrate that culturing ASCs on nonwoven fibers in a 3D bioreactor environment is extremely effective in terms of cell mass production, and alters cell biology in a potentially favorable manner for clinical applications.

Efficient Derivation of Purified Nkx2-1$^+$ Lung and Thyroid Progenitors Via Directed Differentiation of Mouse ES Cells

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During development, specification of progenitor cells that will give rise to the entire parenchyma is an important step in mammalian organogenesis. Although in endodermal tissues, such as the pancreas and liver, the identity of the earliest organ progenitor cells is well defined there is a lack of information regarding the identity and phenotype of the first known primordial lung and thyroid progenitors. Expression of the transcription factor Nkx2-1 is the earliest known marker of these progenitors, however their identity, genetic or epigenetic programs, and signaling

pathways that regulate their cell fate decisions remain largely unknown. Employing a novel Nkx2-1^{GFP} knock-in mouse ES cell line, we derived lung and thyroid progenitors recapitulating defined milestones and lineage commitment decisions of lung/thyroid development. Nkx2-1^{GFP} ES cells underwent endodermal directed differentiation by addition of Activin A, followed by patterning into anterior foregut endoderm by inhibition of BMP and TGFβ signaling, using noggin and SB-431542, respectively. Nkx2-1+ lung/thyroid progenitors were then derived by re-initiation of BMP signaling together with FGF signaling. ES cell-derived Nkx2-1^{GFP+} endodermal progenitors were sorted to purity and retained proliferative capacity in serum-free medium supplemented with FGF2 (250 ng/ml) and FGF10 (100 ng/ml). Nkx2-1+progenitors at the time of their purification represent a primordial endodermal stage prior to induction of mature thyroid or lung genes, but after 7 days of further induction they express markers indicative of thyroid maturation (TSH-R and thyroglobulin), lung epithelial differentiation (SPC and SPB) as well as proximal-distal airway patterning (Sox9, CC10, Foxj1, p63, and CFTR). Furthermore, the cells responded to fetal lung maturation medium, containing glucocorticoids and cAMP, by upregulating ~100-fold expression of surfactant proteins (SPC and SPB). Freshly purified GFP+ progenitors were able to repopulate a 3D decellularized lung scaffold, giving rise to cuboidal alveolar cells retaining Nkx2-1 expression as well as flattened T1α+ cells, with a morphology reminiscent of Type I alveolar epithelial cells. Microarray analysis of GFP+ and GFP- populations at the time of purification allowed us to define the global genetic signature that characterizes the ESC-derived Nkx2-1+ endodermal progenitor population, including 83 novel candidate cell surface markers that are upregulated in the GFP+ population. In conclusion, this novel system and tools allows capture in culture of Nkx2-1+ endodermal primordial progenitor cells of an early developmental stage that occurs only transiently *in vivo*.

SESSION 6: VASCULARIZATION

Chairs: Kara McCloskey (University of California, Merced) and Ying Zheng (University of Washington)

Invited Presentation

The Stromal Role of Mesenchymal Stem Cells In Angiogenesis

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Adult mesenchymal stem cells (MSCs) can be harvested from multiple distinct tissues, such as the bone marrow and the adipose tissue. Transplantation of MSCs has been shown to promote cardioprotection and angiogenesis, however, it appears that this is not necessarily due to the endothelial differentiation of the MSCs. Instead, MSCs secrete multiple paracrine factors that allow them to create a stromal niche that promotes angiogenesis by resident or co-transplanted endothelial cells. This presentation will discuss novel paracrine factors released by MSCs that promote angiogenesis as well as the metabolic response of MSCs that may allow them to increase the survival of co-transplanted endothelial cells. Understanding the stromal function of MSCs may significantly enhance the survival of transplanted regenerative cells by either co-transplanting MSCs or the paracrine factors released by MSCs.

Specialized Tip- and Phalanx-Like Endothelial Cells Derived From Embryonic Stem Cells

Alicia Blancas, Quantitative and Systems Biology, University of California, Merced, Merced, CA and ***Kara McCloskey****, School of Engineering, University of California, Merced, Merced, CA*

Endothelial cells (EC) generated *in vitro* from stem cells are desirable for their potential in a variety of cell-based therapeutic approaches. In this study, distinct EC subpopulations are generated from embryonic stem cells (ESC): the first subpopularion resembles proangiogenic migrating “tip” EC, while the second appears to be composed of purified “phalanx” EC typically found lining vessel lumen. Both ESC-derived EC subpopulations arose from outgrowths of Flk-1+ vascular progenitor cells in our chemically defined medium formulations. However, the phalanx-like ESC-EC are selected early in differentiation for “cobblestone” shape, while the others do not undergo any further selection processing. Here, we show that the unselected “angiogenic” endothelial cells (ESC-AEC) exhibit increased levels of *in vitro* angiogenic sprouting activity compared with ESC-EC. Moreover, the ESC-AEC demonstrate increased migration in response to angiopoietin-2, increased numbers of F-actin stress fibers and fiber organization, and increased HSP27 phosphorylation (related to cell migration). The ESC-AEC also exhibit lower numbers of Flt-1 and Tie-1 positive cells compared with the phalanx-type ESC-EC, while expressing the same high levels of Flk-1 and VE-cadherin. Based on this data, we conclude that the “angiogenic” ESC-AEC are consistent with EC populations that would include the specialized “tip” EC found at the leading edge of sprouting blood vessels, while the ESC-EC represent the Flt-1 expressing, but less migratory, “phalanx” EC found lining the lumen of blood vessels. Perhaps most intriguing is that the presence of these specialized and functionally distinct EC (tip, stalk, and phalanx) subtypes in embryonic stem cell-derived EC cultures challenges the current thought of these EC types only based on their morphological position within the sprouting blood vessel.

Vascular Morphogenesis of Adipose-Derived Stem Cells Is Mediated by Heterotypic Cell-Cell Interactions

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INTRODUCTION: Adipose-derived stromal/stem cells (ASCs) hold great promise as an autologous cell source for regenerative medicine due to their high abundance, ease of harvest via liposuction, and multipotency [1]. In addition to their well-studied potential towards the classic mesenchymal lineages (bone, cartilage, fat), ASCs possess vascular properties and can give rise to both endothelial and perivascular cells [2,3]. Each of these populations is essential to form stable vascular networks. Heterogeneity in adipose-derived stromal/stem cell

(ASC) cultures can give rise to novel cellular interactions, which may be useful for regeneration of complex tissues comprised of multiple cellular phenotypes. More recently, it has been shown that ASCs can spontaneously differentiate in 3D hydrogels to form vascular tubes *in vitro* [4]. However, the key factors that drive ASCs towards the endothelial lineage and vascular morphogenesis still remain unclear and the importance of cell population heterogeneity – which appears to play an integral role in this morphogenic process – is an under-explored concept in tissue engineering.. In this study, we investigated the mechanism by which ASCs undergo vascular morphogenesis in 2D and 3D by examining cellular interactions and phenotypic subpopulations.

METHODS: Human ASCs were isolated from adipose lipoaspirate as previously described [5]. For 2D studies, ASCs were seeded at 20,000 cells/cm2 in endothelial induction medium (EIM; Endothelial Basal Medium-2, 2% FBS, 1% Pen/Strep, 1 µg/ml ascorbic acid, 2 ng/ml VEGF, and 10 ng/ml bFGF) and cultured up to 14 days to monitor vascular morphogenesis. Subsequent cultures were grown in the presence of Tyrphostin AG1295, an inhibitor to platelet-derived growth factor (PDGF) receptor activity to determine whether this signaling was playing a role in the vascular assembly. ASCs were also cultured under either normoxia (20% O2) or hypoxia (2% O¬¬2) for up to 14 days. For 3D studies, ASCs were aggregated into multicellular spheroids via the hanging drop method and cultured in suspension in agarose-coated dishes. In all studies, vascular morphogenesis was assessed via immunocytochemistry for vascular markers (e.g. CD31 and alpha smooth muscle actin (aSMA)).

RESULTS & After 14 days of culture, ASCs self-assembled into complex 3D vascular structures. This phenomenon was only apparent when ASCs were seeded at high density and occurred through orchestrated interactions among three distinct sub-populations: CD31 positive cells (CD31+), α-smooth muscle actin positive cells (αSMA+), and cells unstained for both of these markers (CD31-/αSMA-). Investigations into the kinetics of the process revealed that endothelial vessel-like structures initially arose from individual CD31+ cells through proliferation and their interactions with CD31-/αSMA- cells. During this period, αSMA+ cells proliferated and appeared to migrate toward the vessel structures, eventually engaging in cell-cell contact with them after one week. By two weeks, the lumen-containing CD31+ vessels grew greater than a millimeter in length, were lined with vascular basement membrane proteins, and were encased within a dense three-dimensional cluster of αSMA+ and CD31-/αSMA- cells. Recruitment of αSMA+ cells was largely due to PDGF signaling, as inhibition of PDGF receptors substantially reduced αSMA+ cell growth and vessel coverage. Additionally, we found that while hypoxia increased endothelial gene expression and vessel width, it also inhibited the growth of the αSMA+ population. Recent multicellular spheroid studies have demonstrated that ASCs may utilize the same mechanism to assemble lumen-containing vascular structures within a 3D environment. Ongoing studies are exploring the ability to form these vascular tubes in conjunction with other differentiated phenotypes.
DISCUSSION: This study demonstrates that ASC populations are capable of spontaneously self-assembling to form long, three-dimensional endothelial tubes with lumens and invested by perivascular cells. The heterogeneity of ASCs plays an essential role, as multiple subpopulations appear to be coordinating these events. This self-assembly only occurs when ASCs are seeded at a high density (20000 cells per cm2). These findings indicate that paracrine factors and direct physical interactions among subpopulations may be critical. It also seems that signaling and direct cellular interactions at the earliest stages of culture may be crucial: cells seeded at 10000 cells per cm2eventually attained the same final cell density but do not exhibit similar aggregate formation as cells grown at 20000 cells per cm2.

Intriguingly, the various sub-populations of ASCs appeared to recapitulate some aspects of classic vasculogenesis. In the first few days after seeding, CD31+ cells elongated and proliferated to form multicellular vessel-like structures, while the proliferating aSMA+ cells were initially spatially independent. As the vascular structures elongated, both aSMA+ and CD31-/aSMA- cells appeared to migrate towards the vessel, forming dense clusters of cells around the vessel stalks. These cells fully enveloped the vessel, such that the endothelial cells within experienced a three-dimensional microenvironment. The vessel tracks were lined with vascular basement membrane proteins, collagen IV and laminin, and the vessels themselves exhibited classic morphology, with a patent lumen, thin multicellular endothelial wall, and cells at sprouting tips containing numerous filopodia.

Our studies using the AG1295 inhibitor demonstrate that PDGF-B signaling plays a crucial role in the proliferation and migration of the aSMA+ subpopulation towards the growing endothelial vessels. With the addition of a selective inhibitor of PDGFR to the culture medium, this specific mode of communication between the CD31+ and aSMA+ cells in the culture was effectively blocked and ultimately resulted in thinner vessels and a substantial reduction of aSMA+ cell vessel coverage. This spontaneous organization of endothelial and pericyte-like populations from ASCs has significant implications for their therapeutic applications. The key to facilitating long-term, stable vascular regeneration may lie in the ability to recruit perivascular cells to endothelial networks to provide support and vasoresponsive properties. ASCs, it seems, may be able to perform both roles concurrently through spontaneous assembly of endothelial and pericyte sub-populations; however, seeding cell density appears to be a critical variable influencing this outcome. The investment of vessels by perivascular cells in tissues is a multicomponent process. The formation of vascular structures here suggests that the heterogeneous ASC culture is supplying these or other similar factors that produce the coordinated multicellular morphogenesis.

We also investigated the effects of prolonged hypoxia on cellular responses. Our current study confirmed that under prolonged hypoxia, and additionally demonstrated a thickening of vascular structures, likely due to a VEGF-induced increase in endothelial cell proliferation. Another novel finding in this study was that while prolonged hypoxia appeared to enhance the endothelial subpopulation, it had a negative effect on pericyte growth and vessel coverage. Within the first week of hypoxic exposure, the overall growth of aSMA+ cells in the culture halted, resulting in significantly less co-localization of aSMA+ cells with endothelial vessels compared to normoxic samples by Day 14. While expression of PDGF-B increased in hypoxia is somewhat delayed, and may not be sufficient to overcome the seemingly inhibitory effects of hypoxia in the studied time frame. Inhibition of PDGFR activity under hypox-

ia further reduces aSMA+ cell growth to levels similar to day 0. Taken together, the results of the current study indicate that the effects of hypoxia on the vascular properties of ASCs may be more complex than our previous understanding.

The major barriers to cultivating complex tissues *in vitro* are the incomplete understanding of the kinetics of cellular differentiation that result in stable, terminally-differentiated phenotypes and of the subsequent cross-talk and synergy that arises between these newly differentiated cells and neighboring stem/progenitor cells. This study demonstrates the remarkable ability of a single population of ASCs to spontaneously Ultimately, it may affect clinical application as the ability to harvest all of the necessary cells from a single procedure could potentially reduce cost and recovery time for the patient. Nevertheless, additional studies are required to address these considerations.

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Regulating the Formation of 3D Vascular Networks

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The induction of functional vascular networks has the potential to improve treatment for vascular diseases and to facilitate successful wound healing and transplantation of tissue-engineered organs. Hydrogels are similar to the native ECM of many tissues due to their three-dimensional structural and mechanical properties. In fact, understanding the role of the ECM in vascular morphogenesis has been possible using natural hydrogels as *in vitro* models to study the underlying molecular mechanisms. However, little is known about vascular morphogenesis in synthetic matrices where properties can be tuned, towards both the basic understanding of tubulogenesis in modular environments and as a clinically relevant alternative to natural materials for regenerative medicine. We show how synthetic, tunable polysaccharide hydrogels can be utilized to determine physical and biological parameters that enable efficient formation of functional vascular networks.

One type of hydrogel enables vascular network formation from human endothelial colony-forming cells (ECFCs) *in vitro*. We investigated synthetic, tunable hyaluronic acid (HA) hydrogels and determined both the adhesion and degradation parameters that enable human ECFCs to form efficient vascular networks. Entrapped ECFCs underwent tubulogenesis dependent on the cellular interactions with the HA hydrogel during each stage of vascular morphogenesis. Vacuole and lumen formed through integrins α5β1 and αVβ¬3, while branching and sprouting were enabled by HA hydrogel degradation. Vascular networks formed within HA hydrogels containing ECFCs anastomosed with the host's circulation and supported blood flow in the hydrogel following transplantation. Altogether, we show that the signaling pathways of vascular morphogenesis of ECFCs can be precisely regulated in a synthetic matrix, resulting in a functional microvasculature useful for the study of three-dimensional vascular biology and towards a range of vascular disorders and approaches in tissue regeneration.

Another type of customized hydrogel promotes remarkable endogenous neovascularization and complete skin regeneration. Here we hypothesized that dextran-based hydrogels can serve as an instructive scaffold to promote neovascularization and skin regeneration in third degree burn wounds. Dextran hydrogels are soft and pliable, offering opportunities to improve the management of burn wound treatment. We first developed a procedure to treat burn wounds on mice with dextran hydrogels. In this procedure, we followed clinical practice of wound excision to remove full-thickness burned skin, and then covered with the dextran hydrogel and a dressing layer. Our procedure allows the hydrogel to remain intact and securely in place during the entire healing period, thus offering opportunities to simplify the management of burn wound treatment. A three-week comparative study indicated that dextran hydrogel promoted dermal regeneration with complete skin appendages. The hydrogel scaffold facilitated early inflammatory cell infiltration that led to a rapid degradation of the hydrogel scaffolds, promoting the infiltration of angiogenic cells into the healing wounds. Endothelial cells homed into the hydrogel scaffolds to enable neovascularization by day 7, resulting in an increased blood flow significantly greater than treated and untreated controls. By day 21, burn wounds treated with hydrogel developed a mature epithelial structure with hair follicles and sebaceous glands. After five weeks of treatment, the hydrogel scaffolds promoted new hair growth and epidermal morphology and thickness similar to normal mouse skin. Collectively, our evidence shows that customized dextran-based hydrogel alone, with no additional growth factors, cytokines, or cells, promoted remarkable neovascularization and skin regeneration and may lead to novel treatments for dermal wounds.

Keynote Presentation

Robert Deans
Dr. Deans is responsible for regenerative medicine technology development at Athersys Inc. and its European subsidiary, ReGenesys. Athersys is developing cell therapeutics based on adherent stem cells (MultiStem) isolated from adult bone marrow. Athersys has active Phase I and II clinical development activity in acute myocardial infarct, stroke, ulcerative colitis, and for adjunctive therapy of allogeneic bone marrow transplant.

Dr. Deans has more than 20 years of experience in stem cell therapeutics, having previously served at Osiris Therapeutics as VP of Research. Dr. Deans was previously Director of R&D at Baxter Healthcare, where he developed biological components of the Isolex300i hematopoietic stem cell purification platform. In addition, Dr. Deans served on the faculty at USC Medical School from 1984 to 1992. He holds degrees from MIT and the University of Michigan, and postdoctoral training in molecular immunology at UCLA.

SESSION 7: STEM CELLS IN TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Chairs: Todd McDevitt (Georgia Institute of Technology) and Cynthia Reinhart-King (Cornell University)

Invited Presentation

Human Embryonic Stem Cells and An Engineering Approach to Heart Repair

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This talk will focus on biomaterials-based control of human embryonic stem cells (line H7) differentiated to the cardiac lineage and how these cells, delivered in a unique scaffold, are promising for repair of heart wall infarcts. The scaffold is designed to have the following characteristics: (1) align the cardiomyocytes facilitating formation of muscle bundles that are electrically and mechanically interconnected, (2) minimize cell loss from the heart upon injection, (3) aid in heart integration by rapidly stimulating angiogenesis and inhibiting fibrosis, (4) facilitate placement directly into infarct granulation tissue, (5) the polymer biodegradation can be "tuned" – when heart integration is complete, the scaffold should be completely adsorbed, (6) the synthetic polymer of the scaffold is highly reproducible compared to natural tissue-type scaffolds and it is of similar mechanical characteristics to heart wall, (7) the implant, during static culture conditioning, shows spontaneous purification of cardiomyocytes to greater than 95% purity, (8) cardiomyocytes proliferate in the scaffold after loading thus increasing cell density, (9) mechanical conditioning leads to better cell alignment and possibly olyploidy of the immature cardiomyocytes, (10) the cardiomyocytes in the scaffold beat strongly and show electrical propagation, (11) the polymer class is recognized by the FDA thus simplifying the path to approval.

Patterning Biochemical and Structural Cues Into Collagen-GAG Scaffolds to Alter Mesenchymal Stem Cell Bioactivity for Tendon Insertion Regeneration

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New tissue engineering strategies are necessary to meet a significant clinical need to regenerate spatially-ordered, multi-tissue structures. Orthopedic interfaces in the musculoskeletal system such as the tendon-bone-junction (TBJ) are a prototypical class of such heterogeneous tissues. The TBJ contains a graded interfacial zone linkingtendinous and osseous compartments that is critical for its mechanical competence but that is also a common injury site. Current surgical strategies forsake interface regeneration for only the mechanical fixation of tendon to bone. Our goal is to drive multi-lineage mesenchymal stem cell (MSC) differentiation in a spatially-selective manner within a three-dimensional collagen-GAG (CG) scaffold. We have developed fabrication processed to control regional alignment, mineral content (mineralized CG: CGCaPscaffold), and mechanics within CG scaffolds as well as liquid-phase (diffusible) and solid-phase (immobilized) biomolecule (growth factor, cell regulatory protein) supplementation strategies to mimic the structural and biochemical heterogeneity of the TBJ. Scaffold anisotropy, pore size, mineralization, and soluble biomolecule supplementation have been shown to regulate tenocyte bioactivity and morphology as well as MSC lineage specification towards, and long-term maintenance of, distincttendinous vs. osseous phenotypes [1,2]. Composite biomolecule supplementation strategies utilizing sequestered or freely soluble factors may be particularly suitable for regenerative medicine applications by driving simultaneous proliferation (i.e. IGF-1) and lineage specification (i.e. GDF-5 for tenocyte lineage specification) [3]. Ongoing work is investigating the influence of the degree of GAG-sulfation in the CG scaffold network as a regulator of transient biomolecule sequestration and release as well as determining the potential of coincident matrix and biomolecule cues to drive regionally-distinct MSC differentiation and tissue biosynthesis in a single CG scaffold.

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Expansion and Differentiation of Human Pluripotent Stem Cells In Stirred-Suspension Microcarrier Culture

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Human pluripotent stem cells (hPSCs) differentiated along particular lineages can serve as sources cellular material for regenerative medicine and tissue engineering applications. Realization of the hPSC therapeutic potential necessitates their large-scale generation in a robust and reproducible manner. We adopted the stirred-suspension bioreactor platform for propagating hPSCs in a chemically defined environment under self-renewing

and directed differentiation modes. For microcarrier culture, there are several issues which need to be tackled before this culture modality can be adopted in stem cell olyploidy ng. The low adhesion of hPSCs on the beads is a significant bottleneck. We identified optimal conditions and compared to our previous methodology of seeding hPSC clumps. The efficiency of microcarrier seeding increased from 30% to over 70% with uniform distribution of cells on microcarriers leading to aggregate-free cultures. Moreover, beads were previously coated with mouse tumor-derived Matrigel to facilitate the adhesion of hPSCs. Recently, we applied xeno-free coating promoting hPSC adhesion on the microcarriers and have monitored their growth and pluripotency status in bioreactor cultures. The concentration of cultured hPSCs under xeno-free conditions increased 25-30 fold over 6 days and hPSC pluripotency was ascertained by qPCR, immunostaining and flow cytometry. When hPSCs were subjected to differentiation toward mesodermal and endodermal lineages, the progeny displayed characteristic markers. Our studies support the use of stirred-suspension microcarrier bioreactors for the scalable expansion and differentiation of hPSCs under chemically defined conditions.

Creation of Cell Sheet-Based Bioengineered Heart Tissue Using ES/Ips Cells-Derived Cells

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The bioengineered functional heart tissue is expected to not only function for repairing the broad injured heart, but also be the heart tissue models. We have developed the cell sheet-based bioengineered vascularized heart tissue, however the system to collect the enough amount of cells from ES/iPS cells and the function of ES-derived heart tissue remain elusive. Recently we have established the cultivation system with the suitable conditions for expansion and cardiac differentiation of mouse ES cells and human iPS cells via embryoid body formation using three-dimensional bioreactor with the continuous perfusion system. After the differentiation and purification steps using cells that express some drug resistant genes under the control of aMHC promoter, a large amount of cardiomyocytes were collected. The co-culture of ES-derived cardiomyocytes with the appropriate number of primary cultured fibroblasts on the temperature-responsive culture dishes enabled to form the cardiac cell sheets. Consistent with the findings that cardiomyocytes in cell sheets beat spontaneously and synchronously, connexin43 was expressed at the edge of the adjacent cardiomyocytes and the action potential propagation was observed between cell sheets. Furthermore when ES-derived CD31 positive endothelial cells were co-cultured with ES-derived cardiomyocytes and fibroblasts, robust vascular network formation was observed in the whole cell sheet area. These findings indicate that the development of the bioreactor-based culture system of ES/iPS cells might enable us to collect sufficient amount of cells to create the cardiac cell sheets and the vascular network derived from stem cells in cell sheets might be able to create the thickened heart tissue for the cardiac graft and the heart tissue models.

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SESSION 8: IMMUNITY, INFLAMMATION, AND FIBROSIS

Chairs: Ed Botchwey (University of Virginia) and Sharon Gerecht (Johns Hopkins University)

Invited Presentation

Identification of Molecular Modulators of Cell-Biomaterial Interactions

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The implantation of biomaterials, including tissue engineered constructs, into soft tissues can lead to the development of the foreign body response (FBR) that can interfere with the function of the implant. In general, due to the FBR a largely avascular and dense collagenous capsule forms around biomaterials and scaffolds. A hallmark of the FBR is the formation and persistence of macrophage-derived foreign body giant cells (FBGC) on the surface of the implant. In addition, FBGC have been shown to cause extensive surface damage to a variety of biomaterials and cause the release of microparticles that can have toxic effects. Despite the prominence of FBGC at implantation sites, their formation is not fully understood due to the limited proliferative capacity of isolated macrophages. To this end, we have undertaken various approaches including engineering hematopoietic stem cell niches to expand macrophages. Through our work, we have identified several intracellular and extracellular modifiers of various aspects of FBGC formation including a matrix-degrading enzyme (MMP-9), and a chemokine (MCP-1). More recently, we have identified a new mechanism of biomaterial-induced activation of macrophages involving the inflammasome components Asc1 and Caspase-1. We also show that modulation of the expression or activity of these molecules can lead to enhanced biocompatibility by preventing damage and extending the lifespan of implants.

Host Response and Vascularization of hESC-Derived Engineered Cardiac Tissue In a Rat Model of Myocardial Infarction

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We have previously demonstrated engraftment of scaffold-free human embryonic stem cell (hESC)-derived cardiac tissue

patches in the uninjured heart of an athymic rat. "Tri-cell" cardiac tissue patches containing hESC-cardiomyocytes, human endothelial cells, and stromal cells enable rapid formation of human CD31-positive lumens that anastomose with the rat host vasculature and contain erythrocytes at one week. However, the presence of CD68-positive macrophages in the engrafted patch suggests ongoing inflammation or tissue remodeling. We implanted cardiomyocyte-only patches or tri-cell patches (with hESC-derived cardiomyocytes, human umbilical vein endothelial cells, and mouse embryonic fibroblasts) in a permanent occlusion model of myocardial infarction. At four weeks, beta myosin heavy chain-positive cardiac grafts were present in all implants with sparse human CD31+ lumens in tri-cell patches. Rat endothelial cell antigen (RECA)-positive host vessels infiltrated both cardiac-only and tri-cell patches, albeit at low density. CD68+ macrophage density remained high at 4 weeks and macrophages identified by morphometry were both hCD31+ and RECA+, suggesting regression of neovessels in the engrafted engineered tissue. CD68+ macrophage density was high in engrafted patches, moderate in the collagenous scar, and low in remote uninjured cardiac tissue. Interestingly, scar thickness was increased in both cardiac-only and tri-cell patch hearts versus sham. At 12 weeks after implantation, no hCD31+ vessel lumens were found in tri-cell patch implants and yet macrophages and host vessels remain. This preliminary data suggests that the host orchestrates significant tissue remodeling through inflammation and macrophage activity and forms vessels in engrafted scaffold-free engineered cardiac tissue.

Depletion of SIRT1, but Not SIRT2, Inhibits PMA-Stimulated Megakaryocytic Differentiation of the K562 Cell Line

*Mark T. Duncan, Chemical and Biological Engineering, Northwestern University, Evanston, IL and **William M. Miller**, Chemical and Biological Engineering Department, Northwestern University, Evanston, IL*

It has become increasingly evident that reversible lysine acetylation is important for the regulation of many cell processes. Several thousand acetylated proteins have been identified, and these are enriched for proteins involved in multi-protein, protein-RNA, and/or protein-DNA complexes. Sirtuins (SIRT1-7 in humans) are class III NAD-dependent histone/protein deacetylases that have been implicated in modulating the differentiation of many cell types including adipocytes and endothelial, neural, and skeletal muscle cells. Previously, we showed that the pan SIRT inhibitor nicotinamide and the SIRT1/2 inhibitor cambinol greatly enhance polyploidization and proplatelet formation by human megakaryocytic (MK) cells derived from CD34+ cells in culture.

We are further investigating the roles of SIRT1 and SIRT2 in MK differentiation using the K562 cell line. Addition of phorbol 12-myristate 13-acetate (PMA) induces K562 cells to express the MK antigen CD41 and exhibit morphological characteristics of MK maturation, including enlargement of cell size, polyploidization, and the appearance of cytoplasmic vacuoles. Analysis by immunofluorescence microscopy revealed that, prior to PMA stimulation, K562 cells expressed SIRT1 in both the nucleus and cytoplasm, while SIRT2 expression was not readily detected. Two days post-PMA stimulation, K562 cells still expressed SIRT1, but it was largely excluded from the nucleus. Furthermore, we detected SIRT2 in the nucleus and cytoplasm after PMA addition. Silencing of SIRT1 using shRNA-expressing lentivirus substantially reduced the percentage of CD41+ and olyploidy (≥ 8N) cells. SIRT2 silencing did not appear to alter MK differentiation. However, SIRT2 silencing did promote stronger cell adhesion after PMA addition (consistent with a previous report in HEK293 cells). Finally, a preliminary study examining SIRT1 and SIRT2 silencing in primary MK cells (derived from CD34+ cells) suggests a potential role for SIRT1 in the commitment and/or expansion of MK progenitors because depleting SIRT1, but not SIRT2, decreased the number of CD41+ cells in culture. We are currently following up on these observations.

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Microstructural Controls to Guide Tissue Growth in vitro and *in vivo*

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Formation of new blood vessels represents a key stage of many biological processes, pathologies, and therapeutic strategies. In particular, in the context of regenerative medicine, a scaffold with fast and direct guidance of vascularization is desirable to enhance the tissue growth in poorly vascularized regions or spatially differentiated tissue. However, existing culture systems and tissue scaffolds lack crucial controls of the physiological microenvironment – e.g. concentrations of soluble biochemicals, mechanical stresses, and extracellular structures. In this study, we will discuss challenges and opportunities presented by combining conventional microfabrication with tissue engineering approaches both *in vitro* and *in vivo*.

1. Engineer microvessel *in vitro*. Microvascular networks support metabolic activity and define microenvironmental conditions within tissues in health and pathology. Recapitulation of functional microvascular structures *in vitro* could provide a platform for the study of complex vascular phenomena, including angiogenesis and thrombosis. We have engineered living microvascular networks in three dimensional tissue scaffolds and demonstrated their biofunctionality *in vitro*. We will describe the lithographic technique used to form endothelialized microfluidic vessels within a native collagen matrix; we will characterize the morphology, mass transfer processes, and long-term stability of the endothelium; we will elucidate the angiogenic activities of the endothelia and differential interactions with perivascular cells seeded in the collagen bulk; and we will demonstrate the non-thrombotic nature of the vascular endothelium and its transition to a pro-thrombotic state during an inflammatory response. The success of these microvascular networks in recapitulating these phenom-

ena points to the broad potential of this platform for the study of cardiovascular biology and pathophysiology.

2. Microfabricate tissue templates *in vivo*. Tissue templates for reconstruction and regeneration *in vivo* have achieved clinical successes for homogeneous tissues in well vascularized regions. Outstanding challenges exist for applications in poorly vascularized regions and for spatially differentiated tissues. Here, we present a strategy to control the spatial patterns of cell and vascular ingrowth throughout the volume of a bioremodelable and resorbable matrix via well-defined micropores and networks of microchannels. Our presentation of this approach includes: a description of a lithographic technique to form deterministic microstructures within a matrix of native collagen; elucidation of multistep process by which microstructures drive rapid invasion and vascularization; and demonstration of long range guidance of invasion through the full thickness of patterned templates. Experiments were performed in a murine wound model. These microstructured tissue templates (MITs) could improve outcomes in reconstructive surgery and open paths to directed regeneration of spatially heterogeneous tissues or organs.

SESSION 9: CLINICAL TRANSLATION OF CELLULAR THERAPIES

Chairs: Jennifer Elisseeff (Johns Hopkins University) and Jane Lebkowski

Invited Presentation

Human Embryonic Stem Cell-Derived Cardiomyocytes Couple with Host Myocardium and Reduce Arrhythmias In a Guinea Pig Infarct Model

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The transplantation of human embryonic stem cell-derived cardiomyocytes (hESC-CMs) has been shown to improve the mechanical function of infarcted hearts in animal models, but concerns remain about the capacity of hESC-CMs to undergo appropriate electromechanical integration following transplantation and the risk of graft-related arrhythmogenesis. This presentation will summarize our laboratory's efforts to address both of these issues. First, to assess the integration of hESC-CMs in normal and injured hearts, we generated a genetically-modified line of hESC-CMs that stably express the protein calcium sensor, GCaMP3. GCaMP3+ hESC-CMs exhibit a robust fluorescent transient with each contractile cycle, and this signal provides a convenient readout of graft activation *in vivo*. GCaMP3+ hESC-CM grafts in uninjured guinea pig hearts produced epicardial fluorescence transients that occurred in synchrony with the host sinus rhythm, confirming reliable 1:1 host-graft coupling. Outcomes were more heterogeneous following transplantation into cryoinjured hearts, but a majority of cryoinjured recipients showed large regions of coupled GCaMP3+ hESC-CM graft. Second, we used this same model to determine whether hESC-CM transplantation is pro- or anti-arrhythmogenic. Compared to cryoinjured hearts receiving non-cardiac hESC derivatives or vehicle alone, hESC-CM recipients showed improved mechanical function, as well as a significantly lower incidence of both spontaneous and induced arrhythmias. Taken collectively, these results support the continued development of hESC-based therapies for both the mechanical and electrical repair of injured hearts, but they also indicate a need for new strategies to further enhance host-graft electromechanical integration.

Correction of Duchenne Muscular Dystrophy by Engineered Nucleases

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Duchenne Muscular Dystrophy (DMD) is the most common hereditary monogenic disease, occurring in about 1 in 3500 male births. DMD is caused by mutations in the gene encoding dystrophin, an essential musculoskeletal protein. The absence of dystrophin leads to muscle weakness and wasting, ultimately resulting in fatal respiratory and cardiac disease. At present, there are no treatments that can effectively address the poor life expectancy and quality of life of these patients. Cell-based therapies with skeletal myoblasts, adult stem cells, and pluripotent stem cells have been under preclinical and clinical investigation for 25 years with limited success thus far. Ideally, autologous cells would be used following genetic correction of dystrophin. However, typical approaches for the addition of a wild-type dystrophin gene to these cells have been limited by several critical safety concerns and practical challenges. Alternatively, a new class therapeutics has recently emerged based on targeted gene editing by designer nucleases. These nucleases can be engineered to target any sequence in the human genome and take advantage of natural DNA repair mechanisms to create desired changes to the target locus. For example, zinc finger nucleases (ZFNs) engineered to knockout the HIV-1 co-receptor CCR5 in autologous T cells or hematopoetic stem cells are currently under investigation in several clinical trials. Other studies have utilized ZFNs to correct genetic mutations associated with sickle cell anemia, X-linked severe combined immunodeficiency, hemophilia B, and alpha-1 anti-trypsin deficiency.

This study utilizes synthetic nucleases to edit and correct the dystrophin gene as a novel potential therapy for DMD. The advantage of this method is that the native dystrophin gene is restored, presumably including all of the major isoforms and functions of dystrophin. We designed ZFNs and TALE nucleases (TALENs) targeted to exon 51 of the human dystrophin gene to create genetic modifications at this mutation hotspot that can correct a significant fraction of all DMD mutations. ZFNs were generated using modular assembly (MA) and Context-Dependent Assembly (CoDA). TALENs were generated using the Golden Gate Assembly method. Five MA-ZFNs, seven CoDA ZFNs, and twelve TALENs were tested for gene editing activity at the endogenous dystrophin gene in human K562 cells and in skeletal myoblasts derived from DMD patients with deletions of exons 45-50 or 48-50 that can be corrected by frameshifts in exon 51. Two MA-ZFNs, three CoDA ZFNs, and four TALENs were highly active as measured by the Surveyor nuclease assay. Corrections of the dystrophin gene were made by restoring the dystrophin reading frame in exon 51 by creating small insertions and deletions by non-homologous end joining, introducing short sequences by homology-directed repair, or deleting large sequences with two pairs of nucleases. Following nuclease treatment, human DMD myoblasts were moved to differentiation conditions, and

restored dystrophin expression could be detected in these cells by Western blot. Clonal cell populations were isolated from the treated DMD myoblasts, and 11/27 clones showed modification of the target site in exon 51. DNA sequencing of the target site in these 11 modified clones showed the expected rate of reading-frame restoration (4/11, 36%). Ongoing studies include analysis of dystrophin expression by these clonal populations in differentiation conditions *in vitro*, as well as expression and localization of dystrophin in the bulk treated cells and clones following transplantation into damaged hind limb muscles of immunodeficient mice. The development of the engineered nuclease technology for correction of dystrophin mutations, rather than typical gene addition approaches, represents a transformative cell therapy for treating DMD.

Engineered Monocytes As a Cell Therapy for Ectopic Calcification

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Ectopic calcification (EC) is the abnormal deposition of calcium salts in soft or hard tissues as a result of disease or trauma. EC occurs in blood vessels, heart valves, joints and sites of amputation, and can lead to debilitating clinical symptoms. There are currently no drugs or cell therapeutics that effectively target EC. A treatment aimed at preventing and/or removing calcification in EC would have enormous health benefits for a wide variety of patients who suffer from this problem. In this study, we report the development of a bioengineered cell therapy approach to control monocyte-derived macrophage differentiation to osteoclasts, the cells responsible for resorbing mineral in bone. Oligomerization of receptor activator of nuclear factor κB (RANK) is known to be essential for osteoclast differentiation from monocyte/macrophage precursors. We engineered a murine monocytic cell line, RAW264.7, to express a fusion protein comprising the intracellular RANK signaling domain and FK506-derived dimerization domains that bind to the chemical inducer of dimerization (CID), AP20187. Virally infected cells containing these constructs were treated with the CID and dose-dependent induction of tartrate-resistant acid phosphatase (TRAP) activity and the presence of multinucleated cells were observed. Furthermore, NF-κB signaling was upregulated in a CID-dependent fashion, demonstrating RANK intracellular signaling. CID-induced osteoclasts had robust mineral resorptive activity in both two-dimensional and three-dimensional *in vitro* resorption assays, demonstrating functional activity. Importantly, the CID-induced osteoclasts died when the CID was withdrawn, providing an efficient on/off switch to control extent of mineral resorption. These studies are the first successful application of CID technology to control osteoclast differentiation, survival, and mineral resorption, providing the basis for future development of an engineered autologous cell therapy to treat EC. (Studies funded by Washington State Life Sciences Discovery Fund)

Salivary Gland Stem Cell Therapy for Radiation-Induced Hyposalivation

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Many patients treated for head and neck cancer develop xerostomia, as a consequence of radiotherapy-induced salivary gland ablation. Recently, we showed in mice that c-Kit$^+$ salivary gland stem/progenitor cells can be cultured as salispheres, and when transplanted can rescue irradiated salivary glands from hyposalivation(1). The aim of our current research is to translate these findings to the clinic.

Like in mice, salispheres could be cultured from human salivary gland tissue biopsies. The cultures could also be propagated *in vitro* for up to 7 generations from single cell levels, using a three-dimensional culture system. Moreover, from these spheres, salivary gland organoids could be grown.

In order to test if cells from human salispheres are able to functionally restore the irradiation-damaged salivary gland, we first studied the *in vivo* regenerative potential of the cells in immune-suppressed NOD scid gamma (NSG) mice. PKH26-labeled cells from human salispheres were transplanted into salivary glands of NSG mice, which had previously been subject to 6 Gy local neck irradiation. Two months after transplantation, PKH26-positive cells were observed as organized foci. From the centre outwards of these foci, a gradual reduction in labeling in cell was evident, implying proliferation, and morphologies resembling those of human salivary acinar and duct cells was observed. These areas expressed aquaporin-5, amylase and cytokeratin proteins, associated with functional salivary glands. Additionally, human Muc5B protein was also detected in saliva collected from mice transplanted with human salisphere-derived cells. In order to optimise this technique as a xerostomia therapy, we then tried to identify a stem/progenitor cell from the salivary gland with the potential to regenerate irradiated salivary glands in the long term. Previous data from our lab using the irradiated mouse model suggested that a stem/progenitor cell population resides within the intact salivary gland of the mouse(1). Indeed, cells expressing the CD24, CD29, CD133, CD49f and c-Kit adult tissue stem cell markers could also be isolated from human salispheres. Interestingly, expression of c-Kit in normal human submandibular salivary glands could also be localised to cells in the large excretory ducts, again in a similar way to in mice. In order to investigate the therapeutic potential of this c-Kit$^+$ putative stem/progenitor cell population, we generated PKH26-labelled c-Kit$^+$ and c-Kit$^-$ cell populations from human salisphere cultures, and transplanted them into the irradiated NSG mouse salivary gland. PKH26-positive foci were detected in c-Kit$^-$ transplants two months after transplantation, containing acinar-like cells, but markedly fewer ductal-like structures, in comparison with transplant containing unsorted cells. In contrast, c-Kit$^+$ transplanted glands contained scattered PKH26$^+$ cells. Saliva from glands transplanted with c-Kit$^+$ cells did however contain human-specific Muc5B protein. The

above data, namely the self-renewal capabilities of the salisphere cells *in vitro*, the transformation in mice into functionally differentiated human salivary gland cells and the secretion of human protein into murine saliva provide evidence for the regenerative potential of salisphere derived cells. C-Kit expression may also be used to enrich for stem cells within the transplanted cell pool, although it is unclear so far whether c-Kit$^+$ cells are capable of regenerating the irradiated mouse salivary gland without support from additional human cell types.

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SESSION 10: TECHNOLOGIES FOR STEM CELL ANALYSIS AND CHARACTERIZATION

Chairs: Ali Khademhosseini (Harvard University) and Shyni Varghese (University of California, San Diego)

Invited Presentation

Hydrogel Platforms to Examine Cell-Cell Signaling In MSC Differentiation

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Mesenchymal stem cells (MSCs) represent an attractive cell choice for regenerative medicine applications due to their ability to differentiate toward multiple musculoskeletal lineages. However, there is a dearth of information on how interactions with native cells, specifically paracrine signaling effects, may influence stem cell fate. A more physiologically representative, 3D *in vitro* co-culture system that allows post-culture separation of segregated cell populations would enhance the ability to study these effects. Therefore, in our laboratory, patterned hydrogel-based systems with enzyme-sensitive adhesives to enable co-culture of multiple cell types followed by on-demand separation for subsequent analysis of cell phenotype were developed.

In particular, separable 3-D co-culture laminates were prepared by laminating PEG-based hydrogels with enzyme-degradable hydrogel adhesives, such as those synthesized with chondroitin sulfate (chondrotinase), alginate methacrylamide (alginase) or the collagenase-sensitive peptide LGPA. Replica moldings with PDMS molds resulted in tri-laminated constructs with two cell populations separated by a relatively thin (<500 um) glue layer. Encapsulated human MSC populations exhibited consistent segregation with well-defined interfaces. Furthermore, constructs could be separated on-demand upon addition of the appropriate enzyme. Cell viability remained high after encapsulation, throughout the culture period, and after laminate separation, with no appreciable decrease in viability over the 14 day culture period.

Subsequently, we applied a version of this platform to co- and tri-culture of primary human MSCs, osteoblasts, and adipocytes and analyzed each cell type separately with qPCR and histochemistry for several mesenchymal lineage markers over 18 days *in vitro*. Distinct expression dynamics for osteogenic, adipogenic, chondrogenic, and myogenic transcriptional regulators resulted within each cell type depending on its culture setting. Incorporating this data into multivariate models produced latent identifiers of each emergent cell type dependent on its co-/tri-culture setting. Histological staining showed sustained triglyceride storage in adipocytes regardless of culture condition, but transient alkaline phosphatase activity in both osteoblasts and MSCs. Taken together, our results suggest novel emergent phenotypes for MSCs, osteoblasts, and adipocytes in bone marrow that are dependent on and result in part from paracrine interactions with their neighboring cell types.

Overall, we have successfully designed and implemented a system for the long-term 3D co-culture of segregated cell populations that remain viable after encapsulation as well as separation. We believe a culture platform such as this, combined with sophisticated data analysis and modeling, offers great potential for a variety of basic cell signaling studies to examine co-culture effects and paracrine signaling on stem cell differentiation and function.

Spatial and Temporal Regulation of Signaling Pathways In hESCs Using Microfluidic Technologies

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During development and regeneration, tissues emerge from coordinated sequences of stem cell renewal, specialization and assembly that are orchestrated by cascades of regulatory signals. This complex *in vivo* milieu is poorly replicable using standard *in vitro* techniques, and in this context microscale technologies offer potential for conducting highly controllable and sophisticated experiments at biologically relevant scales and with real-time insights into cellular responses.

We developed a microbioreactor providing multiple gradients of molecular factors in 2D and 3D cell culture settings. The platform's internal modules are interchangeable to support 2D cell culture in a continuous monolayer, or 2D and 3D cell culture in a microarray setting. The platform is capable of establishing complex sequences of time- and space-resolved gradients in the cell culture space, and of applying fast dynamic changes of environmental signals with versatile, high-throughput operations and imaging compatibility.

Important features of the platform are: (i) generation of multiple concentration gradients, (ii) support of different cell culture settings (continuous or patterned, 2D or 3D), (iii) high throughput (by parallelization of multiple platforms), and (iv) compatibility with on-line imaging and μ-array readers. In addition, we

establish capabilities to (v) apply fast dynamic changes of environmental signals (morphogens in combination to their inhibitors), and to (vi) superimpose other biophysical stimuli over the diffusional gradients.

Our platform comprises a matrix of conical microwells accommodating Embryoid Bodies which are protected from potentially harmful shear forces and exposed to stable concentration gradients generated by an integrated microfluidic platform. The top diameter of the 750µm deep microwells can be varied from 100 to 1000µm, tapering towards the bottom with a ~18° angle. For these experiments we chose a 500µm top diameter for the microwells. 100 µm deep channels connect rows of microwells to the main lateral flow channels.

From a single microbioreactor it is possible to obtain 75 data points, corresponding to 5 concentrations across the gradient with 15 repeats each. Mathematical modeling of flow and mass transport within the bioreactor was used to determine the geometry of the cell culture modules and microfluidic channels allowing fast changes of concentrations of characteristic molecular species. The model predictions were experimentally validated using labeled molecular markers.

A reversible hydraulic sealing between the components is ensured by a clamping device, which couples ease of assembly and retrieval of the cell samples for subsequent analyses. The cell culture area in the assembled platform is optically transparent, allowing on line observations using standard microscopes.

H1-BAR-Venus hESCs driving the Wnt/β-catenin–dependent expression of Venus were used in all experiments and exposed to time- and space-resolved concentration gradients of multiple factors. Wnt3a, Activin, BMP4 and their inhibitors were chosen for being the main player in early stage fate specification and lineage commitment in the developing embryo. Venus expression is used as an immediate readout of the biological kinetics of cellular responses following Wnt regulation of the β-catenin pathway. The explored temporal windows of action are as follows: Wnt3a and BMP4 between differentiation day (d) 1.5 and 3; ActivinA between d2 and d4. Similar patterns of stimulations are used in parallel experiments combining the action of the corresponding inhibitors. Concentrations of all factors vary between 10 and 150ng/mL.

Results will be presented correlating the behavior of differentiating EBs to their exposure to complex time- and space-resolved concentration gradients of mesodermal-inducing morphogens. We hypothesize that the application of complex regulatory patterns combining spatial and temporal gradients of molecular and physical factors to hESC (and potentially iPSc) cultures would provide predictable *in vitro* models of development and disease.

An Automated Microfluidic Stem Cell Bioreactor

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Stem cells are promising candidates for a variety of therapeutic applications because of their ability to differentiate into different mature cell types. In order to enable the development of novel stem cell therapies, it is critical to develop technologies that control the environment of stem cell populations such that we can study and understand the mechanisms governing their growth and differentiation. Physicochemical properties such as temperature, oxygenation, and media osmolarity and biochemical inputs such as growth factors, cytokines, access to nutrients, and accumulation of metabolites affect cell behavior. We present a bioreactor platform that uses microfluidic technology to precisely control the physicochemical and biochemical environment of cells.

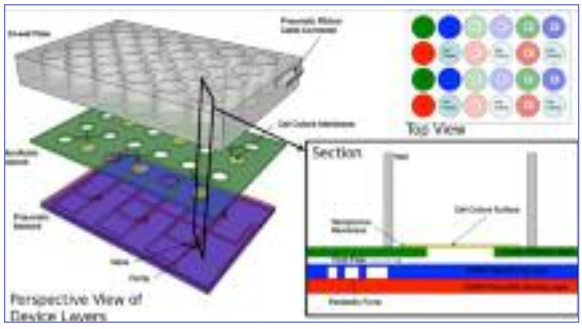

Figure 1 – A perspective schematic of the microfluidic cell culture device is shown with the layers expanded. The section shows that the desired fluid is delivered to the cells from the bottom through a nanoporous membrane on which the cells are cultured. The top view shows the organization of the wells for the reagents and cell culture.

The goal of this project was to fabricate a user-friendly bioreactor platform that could be used by people with no microfluidics or engineering expertise. It has two modules: a disposable microfluidic cell culture unit and a control unit. The cell culture unit is designed using a familiar multiwell plate format featuring microfluidic networks to enable precise delivery of factors to cells. Some of the wells of the the multiwell plate serve the purpose of providing input ports for the microfluidic network and the rest of the wells are used for cell culture. The reusable control unit contains electronic and pneumatic components for driving the microfluidic system. The two units are connected with the pneumatic analog of a ribbon cable.
In the interest of keeping the device operation intuitive and simple, we chose to build the system using a standard multiwell plate format, as shown schematically in Figure 1. A com-

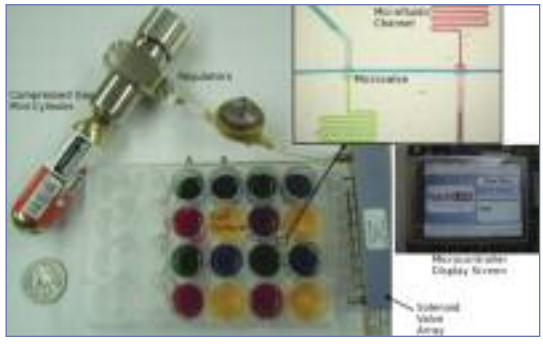

Figure 2 – An optical photograph of a bioreactor microfluidic device prototype is shown with different colors of food coloring used to visualize the wells and underlying microfluidic network. The inset shows an optical micrograph of a portion of the microfluidic channels. The device is operated using a compressed CO2canister and interfaced directly to the miniature solenoid valve array. A photograph of the microcontroller interface is shown at the right.

mercially-available bottomless 24-well plate is chemically bonded to a polydimethylsiloxane (PDMS) microchannel network, which defines the connectivity between the wells. PDMS microvalves are used to control which channels are open at any given time during the experiment. The reagents are circulated between the wells through the channels by means of peristaltic PDMS micropumps. The microvalves and micropumps are fully programmable, such that exchange of factors between wells can be temporally controlled. We designed our device to run three experiments simultaneously in duplicate in a single 24-well plate, as shown in the top view of the device. Each group of four wells has one well for cell culture connected to three reagent wells. The reagent wells are loaded with factors of interest by pipette. The cells are seeded in the cell culture well using any multiwell plate-compatible cell seeding protocol. Microchannel networks with different connectivities can easily be designed and fabricated to enable different types of experiments. A photograph of a prototype device being operated with food coloring for visualization purposes is shown in Figure 2.

The microvalves and micropumps are driven using miniature solenoid valves (part # LHLA_31111H, Lee Company, Westbrook, Ct) Most microfluidics groups operate these solenoid valves via a computer/LabView interface using large tanks of pressurized air, which tethers the device both electrically and pneumatically. We have replaced the computer with a battery-operated microcontroller that can control up to 24 solenoid valves in any desired sequence with millisecond time resolution. Files specifying sequences of valve states are generated on a personal computer using a custom computer application and transferred to the microcontroller on a standard memory card (SD format). The intuitive microcontroller interface allows the user to select and run the desired program from the card. The microcontroller also has inputs to allow data logging from sensors such as thermocouples and pressure sensors (not implemented yet in this generation of the device). The electronics and solenoid valves are powered by a rechargeable battery pack that can be charged using a wall adaptor or by plugging it in to the USB interface of a computer. The unit is capable of operating while the battery charges. Most importantly, the device does not need to be tethered to pressurized air wall intake or a large tank. Instead, we use inexpensive, disposable miniature gas charges as pressure sources. These canisters are less than 3 inches long, under 20 g in weight, and are commercially available filled with a variety of gases for under $2, as shown in Figure 2. They are commonly used for inflating bicycle tires and in whipped cream machines. Because of the small volume of the microscale valves and pumps, a single canister has enough gas capacity to fill millions of valves. The control unit is portable, self-contained, and weighs less than 1 kg. It is straightforward to move the entire experiment to different environments or from an incubator to a microscope without interrupting delivery of nutrients or factors of interest. This feature is important for enabling characterization of live cells. It also opens possibilities for studies of gas environment and temperature on cell growth and differentiation.

In summary, we have developed an untethered, user-friendly bioreactor platform that is ideal for culturing and studying sensitive cell types. Cell populations are cultured in familiar multiwell plates with underlying microfluidic networks to enable precisely timed exchange of reagents without user intervention. Our innovative microfluidic control system makes sophisticated microfluidic technology accessible to facilities without the need for cumbersome and expensive instrumentation.

Keynote Presentation

The Secret Lives of Stem Cells: Genomics and Epigenetics of Human Pluripotent Stem Cells

Jeanne Loring

Jeanne Loring, Ph.D. is Professor and the founding Director of the Center for Regenerative Medicine at The Scripps Research Institute in La Jolla. Her research team primarily focuses on large-scale analysis of the genomics and epigenomics of human pluripotent stem cells (hPSCs) and their derivatives. Most recently, they discovered recurrent epigenetic anomalies in a large collection (200) of hPSC samples. They also recently identified a powerful new biomarker to purify stem cell-derived cells for therapy, generated the first iPSCs from endangered species, and developed PluriTest, a web-based molecular diagnostic test for pluripotency in human cells. Their translational projects include stem cell applications for arthritis, multiple sclerosis, Alzheimer disease, Parkinson's disease, and Fragile X and Rett syndromes, and they are creating collections of iPSC lines for disease modeling and ethnicity-associated drug toxicity. Dr. Loring is also involved in the societal issues associated with stem cell research, including the ethics of stem cell generation and clinical use, the legal implications of stem cell patents, and public education about the dangers of unregulated stem cell treatments ("stem cell tourism"). She serves on the ethics boards of Merck KGaA and the Bill and Melinda Gates Foundation.

1. 3D Cultured Placenta Derived MSC-Like Cells for Cell Therapy – Into the Clinic

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Adherent MSC-like stromal cells (ASC) are favorable candidates for cell therapy, primarily due to their availability and potent secretome. We have shown that placenta derived ASCs induce secretome dependent pro-angiogenic and anti-inflammatory responses in-vitro and in-vivo both in animals and in humans. 12-month data from two Phase I clinical trials have shown that Pluristem's placenta derived ASC product candidate, PLX-PAD, is safe and potentially efficacious in treating patients suffering from Critical Limb Ischemia (CLI). Based on this data it can be said that placental ASCs grown in 3D environment are a promising cell source for treatment of ischemic and immune related diseases. However, production of large numbers of cells for the clinic using 2D culture based platforms has limitations in terms of scalability and process control. To meet the production demand (trillions) of cells that are needed for clinical use we culture ASCs in bioreactors on non-woven fiber carriers. This allows production of large numbers of cells while enabling control over cell culture parameters, and confers to the cells' advantageous biological attributes. To examine the biological effects of our 3D expansion method, placental derived ASC were cultured on 2D tissue culture polystyrene (TCPS), and 3D non-woven fiber scaffolds in spinner flasks and in packed-bed bioreactors. The cells from each growth system were analyzed for ECM deposition, cytoskeleton structure and angiogenic/inflammatory clinically relevant genes and proteins. We found that the biology of ASCs cultured in this 3D method was significantly different from ASCs grown in 2D culture. In 3D culture, ASCs deposit large quantities of collagen and fibronectin, creating an ECM based tissue-like niche. In addition, the cytoskeleton structure observed in 3D differs from that observed in 2D culture where ASCs contain many actin stress fibers. Culture of ASC in the 3D scaffold results in multi-fold up-regulation of angiogenic related genes and >2 fold increase in the secretion of pro-angiogenic proteins as compared with ASCs cultured on 2D TCPS. From the cell expansion perspective, the number of harvested ASCs from one 3D packed-bed (1.5L) bioreactor is equivalent to ASC harvested from 100 2D 10-stack 'cell factory' (Nunc). Taken together, these data demonstrate that culturing ASCs on nonwoven fibers in a 3D bioreactor environment is extremely effective in terms of cell mass production, and alters cell biology in a potentially favorable manner for clinical applications.

2. Adhesive Signature-Based, Label-Free Isolation of Human Pluripotent Stem Cells

***Ankur Singh**[1], Shalu Suri[2], Ted Lee[1], Jamie Chilton[3], Steven Stice[3], Hang Lu[2], Todd McDevitt[4] and Andres Garcia[1]*
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The ability to rapidly and efficiently isolate undifferentiated human pluripotent stem cells (hPSCs) from feeder-cells, somatic cells, or spontaneously differentiated cells is a crucial step towards production of clinical-grade stem cells and relies primarily on manual isolation through morphological assessments or enzymatic methods that cannot differentiate between mixed cell populations. We demonstrate significantly distinct 'adhesive signature' of hPSCs (i.e., hiPSC and hESC) compared to contaminating cells and exploited the differences in adhesion strength to rapidly (<10 min) and efficiently isolate undifferentiated cells as intact colonies from mixed cell population with >98% purity using controlled fluid forces in a high-throughput microfluidic devices. The hPSCs were enriched without adversely affecting the phenotype or karyotype of the pluripotent cells and isolated cells differentiated into the three germ layers. This low-cost, label-free, high-throughput strategy is applicable to a wide range of mixed cell populations with differences in adhesion strength and amenable to several in-line biological analyses towards broader applications in biotechnology.

3. Airbrushing: A Rapid, Low-Cost Method for Fabricating Polymeric Nanofiber Scaffolds for Tissue Engineering

***Wojtek Tutak,** Sheng Lin-Gibson, Tanya Farooque, Jyotsnendu Giri, Dongbo Wang and Carl G. Simon*
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Polymer nanofibers are employed in a wide range of applications including electronics, material reinforcement and biomedical engineering. Non-woven polymeric nanofiber scaffolds are of particular interest to tissue engineering since the fiber mats emulate the structure of native extracellular matrix (ECM). However, nanofiber scaffolds are typically synthesized using an electrospinning method which is slow and requires high voltage equipment. Here we present an alternative airbrushing technique which can be used for rapid nanofiber scaffold fabrication. Fibers are formed when compressed gas (N_2 or air) is mixed with polymer solution and forced through a small nozzle. Airbrushing provides higher deposition rates, safer operation and 100-fold lower cost of implementation in comparison with electrospinning. We show that airbrushing can make nanofiber scaffolds from four different polymers demonstrating its versatility. In-vitro biological evaluation of the airbrushed scaffolds demonstrated their ability to support primary human bone marrow stromal cell growth and osteogenic differentiation. These results demonstrate an inexpensive, safe, high-throughput approach to making nanofiber scaffolds that can be used for tissue engineering applications.

4. Analysis of Cardiomyocyte Aggregates with Time Lapse Phase Contrast Microscopy

***Liew Seaw Jia**[1], Sherwin Ting Qi Peng[2], Chong Wee Keat[1], Tham Jo Yew[3], Li Xiang1, Steve Oh[2]*
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Successful development of human Embryonic Stem Cell (hESC) into Cardiomyocyte (CM) aggregates is perceived as one of the promising approaches in restoring damaged heart muscle cells. Researchers exploring the potential of CM in cell

therapy often need a long-period of observations and statistical analysis of cell development to establish a good understanding of cell differentiation efficiency and yield. A quantitative analysis of CM aggregates has thus been developed to quantify the bioprocess outcome and provide insights into biological behavior over time. The approach begins with video recordings of cells activities in a culture well using phase contrast microscope. The acquired datasets are then translated into important statistical information, such as aggregate size, experimental yield and various beating patterns. In our experiment, analysis shows that hESC development into CM yields 40% or more beating aggregates and suggests that CM aggregate size may not necessarily govern the onset of beating. Furthermore, in order to efficiently manage the gigabytes of video data generated each day, we also developed a scalable multimedia platform that enables automated live video capture from the microscope camera, real-time remote networked video monitoring (on PC and mobile devices) as well as long-term archiving for on-demand search, retrieval and further analysis of the rich CM video library.

5. Analyzing the Spatiotemporal Dynamics of Pluripotent Stem Cell Differentiation In 3D Environments Via Rules-Based Computational Modeling

***Douglas White*[1]**, *Melissa A. Kinney*[1], *Melissa Kemp*[2] *and Todd C. McDevitt*[1,2]
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Pluripotent embryonic stem cells (ESCs) have the unique ability to differentiate into cell types of all germ lineages, making them a potentially robust cell source for regenerative medicine therapies. However, the fate and behavior of ESCs is difficult to control and predict, which currently limits their potential uses in medicine and industry.[1] One of the approaches to induce the differentiation of ESCs is to create multicellular aggregates known as embryoid bodies (EBs).[2] However, this approach fails to provide the degree of control over cell fate necessary for regenerative medicine and stem cell bio-manufacturing applications. Advances in controlling cellular microenvironments have given us a greater degree of control over local cellular microenvironments.[2] Thus, a computational model which can predict phenotypic changes of ESCs in 3-D EBs would be useful in various settings including modeling possible niche-like microenvironments. The objective of this study is to utilize rules based spatial and cellular modeling to provide insight into the underlying mechanisms governing cell fate transitions in 3-dimensional microenvironments experienced by pluripotent stem cells.

EBs were formed by forced aggregation of an Oct4-GFP murine ESCs (D3 line) via centrifugation into 400μm diameter PDMS micro-wells as previously described.[3,4] A range of EB sizes (250, 500, 1000 cells per well) were investigated. After a 24 hour culture period EBs were transferred to a suspension culture and maintained on a rotary orbital shaker at 55 rpm for 11 days.[3,4] Within each size group two treatment groups were established, one in which the cells received leukemia inhibitory factor (LIF) following transfer to rotary culture, and one in which they did not. The size of the EBs was analyzed throughout the 11 day culture. EBs were formalin fixed and stained with Hoechst and Phalloidin to investigate intra-aggregate cellular organization. Pluripotency marker Oct4 was investigated via immunostaining. Stained EBs were imaged using confocal microscopy. Individual confocal images were analyzed to look at cellular packing, organization, and overall connectivity. These metrics were compared to aggregates generated in silico via a forced agglomeration physics simulation and lattice generation methodologies. The lattice generation methodologies provided similar results as the higher resolution physics methodologies in their ability to generate relevant packed physical environments. However, the physics models provided a finer level of control over physically relevant parameters such as cell size, and the number of cells comprising an aggregate. As such, these methods were used to model the creation of EB aggregates in silico. Modeling was accomplished using the following open source software packages integrated into a python shell: vpython (for visualization), pyODE (for physics simulations), matplotlib (graphs and data representation) and escript/finley (partial differential equation solution).[5]

Further modeling was carried out to investigate the patterns formed during the loss of pluripotency in EBs. Three rules were considered in the construction of the pluripotency model: cellular signaling in response to cellular connections, diffusion of soluble factors, and paracrine effects. The strength and interplay of these rules was varied and the effects on the resulting pattern were analyzed. EBs were simulated until 50% of the cells had transitioned out of the pluripotent state. The cellular structures were considered to be static during this time. Initial simulations were performed on the same initial starting structure. Simulations performed with multiple starting structures of similar size and morphology did not show drastically different patterns for a given rule paradigm.

Analysis of the temporal aspects of pluripotency showed that the loss of Oct4 varied based on the number of initial cells used. The smaller the aggregate, the faster the onset of the differentiation events occurred. In cases where confocal images captured an EB with a partially differentiated phenotype, the resulting pattern was classified. Classifications of patterns were identified based off the modeling results from the aforementioned sets of rules. The patterns were consistent with an outside-in loss of pluripotency, however, they were not necessarily always spherical. Often clusters or pockets of Oct4 expression remained tucked within the EBs. This type of pattern was consistent with the dominant paracrine signaling scenario simulated in the model.

These results indicate that the state transition between pluripotency is largely modulated by the local LIF regulatory networks, which agrees with published results[6]. These results validate the strength of this modeling methodology to elucidate the rules governing important cell fate decisions in 3D environments. Due to the structural nature of this model questions relating to changed in structure are easily investigated, as well as perturbations in the local soluble factor gradients. This model can also be extended to investigate what factors influence differentiation into different germ layers, and eventually used to predict optimal niche and microenvironment organization for optimal stem cell differentiation.

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6. Arteriogenic Growth Supports Vascularization of Cardiac Grafts

***Jill Weyers[1]**, Elina Minami[2], Sarah Dupras[1], Kevin Weitz[1], Dara Carlson[1], Timothy Cox[3], Stephen Schwartz[1], Charles Murry[1,4] and William Mahoney Jr.[1]*
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Repairing and replacing injured myocardium is a central goal in the treatment of myocardial infarction. One promising method for achieving this is to engraft cells into the injured area of the heart that will become a functional component of the heart muscle, replacing or preventing the formation of scar tissue. While engraftment of cells into heart infarcts has been achieved, grafts are small, as grafted cardiomyocytes have a poor survival rate due to continued ischemia within the graft and surrounding tissue. If these grafts are to restore mechanical function, they must become vascularized, both to allow paracrine cross-talk between tissue compartments, as well as to ensure the tissue is fully perfused. Previous efforts to revascularize the injured myocardium have focused on angiogenic mechanisms. These attempts, however, have proved insufficient, as angiogenesis provides new capillaries, but capillaries alone cannot efficiently carry blood. Larger vessels are needed to deliver and remove the blood. Thus, a hierarchical vascular network must be established for effective flow within grafted tissue. At present, the development and remodeling of vessels within engrafted tissue to form a functional vascular network is poorly understood.

While cardiomyocyte engraftment is hampered by poor cell survival, engraftment of skeletal muscle myoblasts (C2C12 cells) results in large grafts within the heart. Though these grafts do not mechanically couple with the host myocardium, and therefore are not contributing to heart function, their large size provides an ideal environment in which to study the vascularization of engrafted tissue. As such, we are using a previously established model of murine heart infarction and skeletal muscle myoblasts engraftment to visualize and characterize the growth of vessels within cardiac grafts. Our work reveals that at 28 days post-infarction and cell engraftment, new vessels are present within the grafts. Using both micro-Computed Tomography scanning and histological analysis, we have characterized the vessels that form within these cardiac grafts, determined where the new vessels branch off the pre-existing coronaries, and analyzed the extent to which the graft vasculature is an efficient hierarchical network capable of supporting sufficient blood flow. Our results provide the basis for a new understanding of how engrafted tissue becomes vascularized via growth from the host vascular network.

7. Benefits of Hypoxic Culture On Mesenchymal Stem Cells

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Oxygen is essential for life, but cultivation of cells is usually performed under 21% O2. Such a condition does not replicate the hypoxic conditions of normal physiological or pathological status in the body. Recently, the effects of hypoxia on bone marrow multipotent stromal cells or mesenchymal stem cells (MSCs) have been investigated. In a long term culture, hypoxia can inhibit senescence, increase the proliferation rate and enhance differentiation potential along the different mesenchymal lineages. Hypoxia also modulates the paracrine effects of MSCs, causing upregulation of various secretable factors, including the vascular endothelial growth factor and IL-6, and thereby enhance wound healing and fracture repair. Finally, hypoxia plays an important role in mobilization and homing of MSCs, primarily by its ability to induce stromal cell-derived factor-1 expression along with its receptor, CXCR4. After transplantation into ischemic limb, an environment combined of hypoxia and serum deprivation, can lead to apoptosis or cell death, which can be overcome by the hypoxic preconditioning of MSCs. More importantly, part of the mechanisms involved in the increase of MSC properties by hypoxic culture is through the upregulation of hypoxic inducible factor-1 (HIF-1) signaling pathway.

8. Bioengineering Strategies for the Development of Robust and Integrated Processes for Expansion and Cryopreservation of Human Pluripotent Stem Cells

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The successful transfer of human embryonic stem cell (hESC) technology and cellular products into clinical and industrial applications needs to address issues of automation, standardization and the generation of relevant cell numbers of high quality. Another major challenge is the establishment of integrated bioprocesses capable to guarantee efficient cell storage and distribution after large scale expansion. Such protocols must assure high cell survival, low differentiation rates and maintenance of cell pluripotency after expansion and cryopreservation.

The laborious and time consuming 2D-cultures are difficult to control, present poor yields and often lack the required cell functionality. These characteristics can have severe consequences on robustness, reproducibility, scalability and relevance of the cell systems, hampering their possible application in Cell Therapy. Therefore, our aim was to develop an efficient and scalable 3D-culture system for the production of clinically relevant hESCs by exploiting the potential of bioreactor technology.

By combining microcarrier technology with environmentally controlled stirred tank bioreactors and further on with cell microencapsulation technology, we were able to improve the final cell yield in 15-fold when compared to the standard 2D cultures. This significant improvement was achieved by:

i) Controlling the pO_2 at 30% air saturation rather than at 5% pO_2. Cell metabolism become more efficient, leading to increased hESCs growth rate and maximum cell concentration (3-fold);

ii) Implementation of an automated perfusion system for continuous addition of small molecules. This resulted in accelerated cell growth and higher cell concentrations for longer time periods. The expanded hESCs retained their undifferentiated phenotype and pluripotency (*in vitro* and *in vivo*);

iii) Application of cell microencapsulation technology for improved cell expansion yields (protects cells from the hydrodynamic shear stress and avoids aggregates/microcarrier clumping) and easy bioprocess integration with cryopreservation protocols. Alginate was the encapsulation material used due to its intrinsic properties (e.g. biocompatibility, biosafety and permeability) and to its potential for cell transplantation. The combination of cell microencapsulation with microcarrier technology resulted in a highly efficient protocol for the production and storage of pluripotent hESCs. This strategy ensured high expansion ratios (approximately 20-fold increase in cell concentration) and high cell recovery yields after cryopreservation (>70%). When compared to non-encapsulated cells, an improvement up to 3-fold in cell survival post-thawing was obtained without compromising hESC quality.

The robust integrated bioprocess developed herein represents a significant breakthrough towards the translation of hESCs to clinical and industrial applications

9. Biomimicry of Cellular Interactions in 3D Stem Cell Scaffolds Via Proteolipobead-Matrix Hybrid Systems

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Re-creation of the complex 3-D microenvironment of the stem cell niche in order to elicit regenerative control is one of the major challenges in biomaterials research. The overall aim of our effort is to test the hypothesis that the introduction of biomembrane-microsphere assemblies into 3D scaffolds is a viable biomimetic means to present ligands/bound factors to stem cells and mimic cellular communication in the stem cell niche. We have developed a new platform to present molecules to stem cells in 3D within a biomimetic architecture: as laterally-mobile molecules embedded in the context of a tailored biomembrane. The presentation of ligands/bound factors involved in cell-to-cell interactions (e.g. cadherin) has begun in 2D culture systems by surface patterning but has not yet been well established in 3D stem cell culture systems.

We present results on three aspects of this study: 1) the fabrication and characterization of functionalized microspheres that contain supported lipid bilayers; 2) the construction and characterization of collagen-I/microsphere hybrid scaffolds that integrate tether-supported lipid bilayers on microspheres of various sizes and 3) preliminary studies of human MSCs microencapsulated into matrix/microsphere hybrid scaffolds.

Confocal microscopy studies were conducted to visualize proteolipobead-displayed N-cadherin engaged in interactions with human mesenchymal stem cells (MSCs) in situ, from 3D CLSM reconstruction of hMSCs in N-cadherin proteolipobead/Collagen-I 3D constructs. Immunohistochemical staining was used to localize the N-Cadherin within the hybrid matrices relative to counterstained supported lipid bilayers. In a small fraction of the MSCs interacting with the proteolipobeads (PLBs), images consistent with PLB-to-MSC biomembrane fusion were evidenced. In essence, molecular engineering of the MSC surface was evidenced where N-Cadherin from the engineered PLBs diffused onto the surface of live MSCs. This work constitutes a new method for displaying a wide range of complex membrane proteins and signaling molecules to live cells within 3D matrices. We present further investigations of MSC-PLB interactions under a range of conditions and surface characteristics of the PLB substrates.

10. Cell Line and Stage-Specific Optimization for Enhanced Endothelial Differentiation of Mouse and Human Embryonic Stem Cells

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Both mouse and human embryonic stem cells (ESC) can be differentiated into functional endothelial cells (EC) *in vitro*. Currently, monolayer differentiation is performed by a step-wise process with endothelial specific signals from both extracellular matrix (fibronectin and collagen-type IV) and soluble factors (VEGF, BMP-4, and FGF). Individual ESC human lines (H7 and H9) and mouse (commercially available: E14 and R1, as well as, our own ESC lines: A3 and B2) were examined. Based on the expression of Flk-1/KDR VEGF receptor, we have optimized the differentiation protocols for each of the above ESC lines varying initial seeding density, matrix substrate, and VEGF concentration. The first stage of differentiation of human ESC was found to express maximum numbers of KDR positive cells on days 14 and 12, for H7 and H9 ESC lines respectively. The four mouse ESC lines expressed maximum numbers of Flk-1 positive cells between 2 and 4 days. Although the optimal matrix and VEGF concentration varied slightly between cell lines, the most significant variable for increasing the number of Flk-1/KDR+ cells was found to be density of the initial cell seeding. Most importantly, we found that the extracellular matrix signaling for directing EC fate is conserved between murine and human, however the time period required for development is distinct between the two species.

11. Characterization of Thawed Human Hematopoietic and/or Mesenchymal Stem Cells Using Colony Forming Unit Assay

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Stem cell related regenerative therapy is based on cell banks of cells, a source of cell therapy, or release products for patients affected with incurable diseases. Therefore, transplantation of effective cell therapy is necessary for stable technological approaches related cryopreservation method, quality control, etc. The present investigation was tried to evaluate the stemness ability of the thawed cells. The cells used in this experiment were hematopoetic stem cells (HSC) from human umbilical cord blood or mesenchymal stem cells (MSC) from umbilical cord. Especially, the experiments focused on colony forming unit (CFU) assay of thawed HSC or MSC. Samples were treated a proper protocol in medium and were frozen with controlled rate freezer. HSC was checked CD34-positive cells and rate of CFU-erythroid (E), CFU-G (granulocyte), CFU-M (macrophage), CFU-GM, burst-forming unit (BFU)-E, and CFU-GEMM after short incubation. The groups of MSC were evaluated by CFU-fibroblasts (F) and doubling number in long-term cultivation. In addition, representative marker of stem cells was analyzed by polymerase chain reaction and immunocytochemistry. The results of the present study will provide a fundamental base for parameters of stem cell banks for regenerative therapy.

12. Characterization, Culture and Differentiation of NKX2.5 Derived Cardiac Precursor Cells From the Human Heart

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The culture and expansion of adult human cardiac progenitor cells (ahCPC) is challenging due to the propensity of these cells to lose their stemness with the current stem cell culture conditions and the lack of definitive markers to identify this rare subpopulation of cells. An alternative to standard two-dimensional culture systems are cardiospheres, which are formed by a heterogeneous group of cells isolated from the heart including CPCs. A subpopulation of c-Kit positive has been identified within cardiospheres but whether this c-Kit population accounts for this CPC-like activity is unknown. Our goal is to identify and characterize which population in cardiospheres represents the hCPC. We dissociated heart samples in a suspension of single cells from fetus and adult patients that they were plated in primary monolayer cultures followed into cardiospheres. We utilized expression of the cardiac progenitor factor NKX2.5 to identify potential CPCs. The NKX2.5 population was negative to cardio-myogenic markers (Troponin I, Calponin) but represented heterogeneous population with a variable expression of other progenitor cell surface markers including hematopoietic stem cell markers such as CD34, CD90, CD117; endothelial progenitor markers such as CD31, CD144, VEGFR1 (FLT1), VEGFR3 (FLT-4), VEGFR2/KDR; Mesenchymal and Neural Crest stem cell markers such as CD90, CD271 and mobilizing stem cells markers such as CD114-PE, CD184-PE. Our data also demonstrate that Nkx2.5$^+$ cells are greatly influenced by the 3-D environment of the cardiospheres as there were dramatically different cell populations in primary monolayer adherent culture versus cardiospheres suspension cultures. Thus, primary cardiospheres and/or adherent cultures from fetal/adult human heart samples are comprised of distinct Nkx2.5$^+$ subpopulations with cardiovascular differentiation potential.

13. Controlled Generation of Hematopoietic Progenitor Cells From Pluripotent Stem Cells Using Microenvironmental Cues

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Pluripotent stem cells (PSCs) serve as tools for research into disease models, and may provide a renewable source for cell therapy applications. Realizing this potential requires robust strategies to guide PSC differentiation into functional cells and tissues. We hypothesize that in addition to soluble factors, other signalling components such as matrix ligands and cell-cell contact may direct cell fate and maturation. Definitive blood cell generation during embryonic hematopoiesis is a dynamic and tightly controlled process dictated by various parameters.

Current *in vitro* techniques utilize xenogenic products and lack the ability to replicate the *in vivo* niche thereby displaying primitive blood cell generation from PSC cultures. In order to mimic the embryonic environment for hematopoietic cell generation, we synthesized an artificial stem cell niche wherein vascular endothelial growth factor A (VEGFA) was immobilized in an agarose hydrogel to drive mouse ESCs towards HPCs1. Agarose hydrogels were first modified with coumarin-protected sulfhydryls2, which upon ultra violet (UV) radiation provided reactive thiols for protein attachment. Maleimide-activated VEGFA was immobilized onto agarose thiols because VEGFA has been shown to play an important role in the generation HPCs3. Mouse ESC aggregates were encapsulated in VEGFA immobilized agarose and compared to soluble VEGFA-supplemented controls during 7 days of culture in serum-free conditions. In the presence of bone morphogenetic protein-4 (BMP-4), T+VEGFR2+ expression was 8- and 75-fold higher with 25 ng/ml and 50 ng/ml of immobilized VEGFA respectively, while CD34+CD41+ expression was 8- and 108-fold greater, compared to equivalent concentrations of soluble VEGFA. Both 25 ng/ml and 50 ng/ml immobilized VEGFA treatment groups enhanced blood development when coupled with 5 ng/ml BMP-4, producing 9- and 23-fold more colony forming cells (CFCs) compared to their respective soluble controls.

Building on these results, we have been able to control cell-cell mediated contact both in 2-D and 3-D using microcontact printing[4,5] and AggrewellTM[6] culture respectively. Using the Aggrewell technology, human PSCs were cultured towards HPCs in serum-free conditions. Briefly, Hes2 and H1 PSCs were centrifuged into AggrewellsTM as single cells and incubated in the prescence of rock inhibitor (Y-27632) and blood-inducing cytokines in normoxia for 20 days. Hematopoietic differentiation was assessed by upregulation of blood markers (evaluated by q-RTPCR and flow cytometry) and generation of blood progenitor cells by colony forming unit assays. Both these studies demonstrate the use of tools (functionalized hydrogels and AggrewellTM) that allow us to recapitulate aspects of the embryonic microenvironment to guide PSCs towards HPCs in a scalable fashion.

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14. A Convenient Micropillar-Based Surface to Control the Size and Distribution of Human Embryonic Cell Colonies

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The promise of pluri-potential human embryonic stem cells (hESC) as a self-renewable source that can become any specialized cell type has attracted enormous attention in the fields of cell therapy, tissue engineering, developmental biology and drug discovery/screening. The ability to recapitulate human development *in vitro* is of significant interests. Among the many attempts, embryoid bodies (EB) formed from 3D hESC aggregates in suspension is often employed as an initial differentiation step as EB permits the generation of the three germ layers, ectoderm, mesoderm and endoderm, which give rise to all the cell types in human body.

Embryogenesis is a complex process involving appropriate chemical, mechanical, temporal and spatial cues. Optimizing a well-controlled microenvironment is inevitable in stem cell differentiation research. Studies have suggested that one important factor that may regulate lineage commitment is EB size. However, conventional suspension culture produces EBs heterogeneous in size and morphology. An intuitive concept of using microwell array to produce homogenously sized EBs has been widely demonstrated. However, one of the major drawbacks of this type of design is its low delivery of differentiation cues to the basal surface of attached cells which may result in nutrient deficiency and difficult cell detachment.

Here we present a novel and convenient method utilizing patterned, corona treated, micropillars surfaces to produce uniform-sized hES cell colonies and EBs. The micropillared surfaces were created by standard PDMS molding process against silicon machining masters. The surfaces subsequently underwent corona discharge treatment for 15 seconds to enhance the adsorption of MatrigelTM, a surface coating required for stem cell attachment. Because corona treatment favored the tops of protruding pillared areas, stems selectively attached to these surfaces leading to the controlled formation of stem cell colonies.

The TW6 human embryonic stem cell line, derived by Industrial Technology and Research Institute (ITRI), was used in this study. Stem cells (5x104/ well) were seeded into 24 well plates, where each well contained micropillars of different dimensions. After 5 days of culture, cells formed colonies only on the pillared areas. At day 5, staining for stem cell markers, alkaline phosphatase and Oct-4, verified that the cells were maintained undifferentiated. The colonies size were detached and measured to be 277 (std: 4%), 333 (std: 2%) and 387 (std: 2%) um corresponding to 300, 400 and 500 micropillared areas. The cell numbers of each colony was also obtained to be 1192 (std: 5.3%), 2726 (std: 8%) and 3088 (std: 9%) respectively. The stem cell colonies were harvested and cultured in suspension (Corning Ultra Low Attachment dish) for 14 days to produce EBs. EBs were then re-plated on gelatin coated dishes for 8 days prior to RT-PCR analysis. This analysis demonstrated the expression of endodermal, mesodermal and ectodermalgenes thus confirming that the undifferentiated stem cell colonies were capable of forming EBs comprised of all three germ layers.

In conclusion, corona-treated micropillars provides a convenient, easy-to-handle plate format to produce variable-sized, highly-uniformed hESC colonies and EBs that can be easily harvested (>80% recovery) for a variety of applications.

15. Culture with Human Platelet Lysate Enhances Mesenchymal Stem Cell Physiology

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Introduction: Mesenchymal stem cells (MSCs) are clinically useful due to their immunomodulatory and regenerative properties. However, despite promising pre-clinical and clinical data, several concerns regarding the safety of current MSC therapies remain. To date, the majority of *ex vivo* cell manipulations have relied on fetal bovine serum (FBS) to propagate cells in adherent cultures. Since FBS is xenogeneic, cells exposed to it have the potential to become immunogenic, and it has been shown that repeated administration of cells cultured with FBS can elicit adverse reactions in patients. As a result, even autologous transplanted cells can be rapidly rejected by the host, and several countries have now imposed restrictions on the clinical use of cell products prepared with FBS. Furthermore, although MSCs maintain their plasticity for

several passages in adherent cultures, it has been demonstrated that MSCs eventually lose their ability to self-renew, replicate, and form colonies with successive passages, often long before adequate cell numbers for transplantation can be obtained. Such challenges surrounding MSC culture currently limit their therapeutic potential, and therefore, there is a significant need for improved methods for MSC expansion *in vitro*. Recently, it has been demonstrated that replacing FBS with human platelet-derived growth factors (in the form of human platelet lysate, hPL) can promote rapid MSC growth while maintaining differentiation potential and immunophenotype. However, the effects of hPL on MSC physiology and its implications for the subsequent therapeutic application of these cells*in vivo* remain unknown. Thus, the purpose of this study was to elucidate the effects of hPL on MSC growth, chemokine receptor expression profile, metabolism, and senescence. Given the enriched growth factor content of hPL compared to FBS, it was hypothesized that hPL culture would enhance the aforementioned MSC physiological properties, thereby rendering them more suitable for therapeutic tissue repair and regeneration.

Methods & Results: Adult human bone marrow-derived MSCs were cultured in medium containing 10-16% FBS or 2-10% hPL. Cell viability, doubling time, and cumulative population doublings were determined over 15-20 passages. Early-passage MSCs were collected for flow cytometry analysis of four chemokine receptors: CXCR4, CCR2, CCR5, CX3CR1. In addition, cells were exposed to physiological stress (serum deprivation), and media were collected at each passage to analyze cell metabolism using a BioProfile FLEX analyzer (Nova Biomedical). MSC senescence was assessed via a fluorometric assay for endogenous beta-galactosidase (β-gal) expression coupled with quantitative morphometric analyses of cell size. Furthermore, to evaluate the ability of hPL to "recover" senescent MSCs, FBS MSCs were split at late-passages, and a portion of cells was further cultured in hPL. Although FBS and hPL both supported MSC expansion and no difference in cell viability was evident, hPL resulted in more rapid cell proliferation at earlier passages. Specifically, MSC doubling time was lower in all hPL cultures (2-10%) compared with 16% FBS, thus hPL cultures yielded a greater number of population doublings. Bioprofile analyses demonstrated no marked differences in cell metabolism at early passages. However, after just 6 days of culture, significant differences ($p<0.05$) were observed in the expression profiles of the chemokine receptor CXCR4; hPL MSCs were 67.5 ± 12.6% positive while FBS MSCs were only 20.7 ± 5.8% positive. Conversely, CCR2 expression was observed to be marginally higher in FBS cultures (47.9 ± 6.1%) compared to hPL cultures (40.6 ± 12.3%), while CCR5 and CX3CR1 were highly expressed (>83%) in both FBS and hPL cultures. With continued culture, FBS MSCs stained positive for β-gal and demonstrated differences in cell morphology as early as passage 11, suggestive of the onset of senescence. At all passages examined, hPL MSCs demonstrated less endogenous β-gal production than FBS MSCs. Additionally, hPL cultures contained visibly smaller cells, forming tightly packed networks, whereas FBS cultures were larger and more randomly-oriented. Forward and side scatter flow cytometry data not only confirmed that FBS MSCs were larger than hPL MSCs, but also demonstrated that the population cultured in FBS exhibited a wider scatter plot in both the forward (cell size) and side direction (internal complexity) and thus, was more heterogeneous than the hPL population. However, phalloidin staining of MSCs at early-, middle-, and late-passages demonstrated no appreciable differences in cell cytoskeletal organization as a result of media supplementation. A notable increase in MSC doubling time was evident at passage 13 and persisted beyond this point (average doubling time = 3.23±0.92 days prior to passage 13 versus 10.83±2.99 days after passage 13). By passage 16, MSCs cultured continuously in FBS ceased to proliferate and cell size (average area = 23,786±6828 um2) was significantly increased ($p<0.05$) compared to passage 5 MSCs (average area = 3,489±520 um2). In stark contrast to passage 16 FBS MSCs, passage 16 recovered MSCs (FBS-hPL MSCs) continued to proliferate, and while cell area (average area = 10,856±988 um2) was significantly increased ($p<0.05$) compared to passage 5 MSCs, cell size was significantly smaller ($p<0.05$) in passage 16 FBS-hPL compared to passage 16 FBS cells. In addition, by passage 16, few FBS MSCs maintained a spindle-shaped morphology while more hPL and FBS-hPL MSCs demonstrated this typical MSC morphology. Subtle differences in cell metabolism, waste (lactic acid and ammonium) production in particular, were evident with prolonged culture and upon subjecting cells to serum deprivation or recovering late-passage FBS MSCs in hPL. However, differences in MSC metabolomics were more a function of passage number (i.e. cell age) than media formulation. Interestingly, at all passages examined, FBS-hPL MSCs exhibited less endogenous β-gal production than FBS MSCs after just one passage following recovery. Recovered MSCs also exhibited accelerated growth (decreased doubling time and increased population doublings) when compared to cells maintained in FBS. Flow cytometry analyses for cell size (forward scatter) and internal complexity (side scatter) also revealed differences between FBS and FBS-hPL MSC morphology immediately following recovery. Recovered MSCs were smaller and tended to once again exhibit a more spindle-shaped morphology than cells maintained in FBS.

Conclusions: Taken together, these data confirm that culture of bone marrow-derived MSCs in hPL enhances cell proliferation and attenuates cell senescence compared to FBS culture. In addition, recovery of senescent MSC populations may be possible by exposure to hPL. The results of this study may enable more effective clinical-scale expansion of MSCs than current *ex vivo* culture techniques and may also have implications for the expansion of MSCs from old or diseased patients.

16. Cyclic Mechanical Stretch Affects Membrane Integrity During Myogenesis

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Mechanical stress plays a crucial role in the development of new physiological responses to cell culture micro-environment. It is recognized that extracellular stimuli promote cellular growth and survival, influence metabolic processes (including gene expression), and governs tissue architecture in various cell types (Kumar et al., 2004,FASEB J). Muscle cells are quite responsive to mechanical forces. This is evident by the

fact that expression of several genes and maturation of skeletal muscle can be modulated by applying an external mechanical stress (Liao et al., 2008, Cell Mol Bioeng). In addition, it is well known that topological organization of cell substrates enhances the myogenic differentiation into functional myotubes.

A number of technological solutions have been proposed to apply an isotropic stretch to the cells on elastic silicone membrane by aspirating the membrane. However, in these systems the confocal optical analysis are not straightforward and a proper micro-patterning for muscle differentiation could be hardly achieved.

In this work, we aimed to design an ad hoc cell-stretching device to accurately control cell topology and deformation along biaxial directions. The use of a microfluidic-based technology allows us to achieve confined deformable micro-areas within culture chamber, while maintaining optical observation. Pressurization of microfluidic channels gives an instantaneous and uniform mechanical stimulus to the cell culture. The development of a computational stress-strain model allows us to predict the cell deformation up to 15% by tuning pressure intensity at different frequency from 0 to 1 Hz. In addition, we developed parallel microgrooves to provide topological stimuli for obtaining parallel oriented myotubes. The coupling of a microgroove-technology with the stretch machinery allows performing stretch experiments on highly differentiated myotubes with well-defined sarcoplasmic organization, in order to obtain a biological read-out from applied mechanical stimulation.

Immunofluorescence analysis of cyclically stretched myoblast at 1 Hz shows a remodelling of cytoskeletal actin filaments and reoriented stress fibres along the direction of maximum strain. The same result was not observed by applying a static deformation (15% at 0 Hz), meaning that, in terms of cytoskeletal remodelling, cellular responses to mechanical stretch are frequency-dependent. Human myoblasts were cultured and differentiated into mature myotubes and loaded with a membrane-impermeable dye, in order to test cell membrane integrity. After 30 min of cyclic stretch at 0.1 Hz, fluorescent dye labelled internal membranes of stretched myotubes, meaning that a reversible micro-rupture occurred on cell membranes. Such system could be very useful to derive an in vitro skeletal muscle model for testing cell membrane integrity under mechanical stress conditions. In particular it could be very useful for evaluating the membrane contribution in mechano-transduction hypothesis in both physiological and pathological conditions. Reversible membrane micro-ruptures produced under cyclic stress stimulations were detected for human myotubes derived from both healthy and Duchene muscular dystrophy patients and the crucial role of dystrophin in preserving membrane integrity was evaluated.

17. Density Gradient Multilayer Polymerization: A Facile Method to Create Scaffolds for Culture of Complex Tissue

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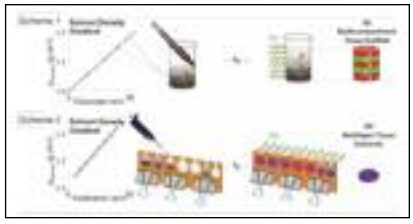
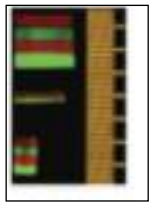
Engineered biomimetic tissue could provide therapies for degenerative diseases or injuries that cause loss of essential functions or an alternative system in which to study fundamental biological questions. Culturing such complex tissue requires scaffolds with multiple compartments of distinct structures and chemical components.[1] Current methods of fabricating such matrices are complex or expensive to implement and often suffer from mechanical weaknesses between layers.[2] Thus, an adaptable, facile, and economical multilayer polymer fabrication technique that produces continuous interfaces between layers is needed. In this presentation, we will describe such a method: density gradient multilayer polymerization (DGMP). DGMP exploits sucrose and iodixanol as aqueous density modifiers to separate the components of each layer, which allows control over the degree of gradation between them.

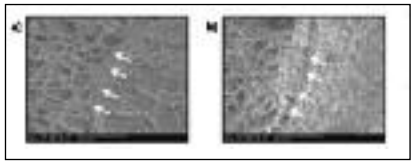


Schematics of DGMP for creating 3D and 2D scaffolds.



Polyacrylamide scaffolds. Ticks= 1 mm.

To create a layered scaffold via DGMP, solutions of either sucrose or iodixanol are first serially diluted. To these separate solutions, the desired concentration of polymer and biological cues are added (Fig. 1). Each solution is gently layered in order of density into a mold; molds for 3D scaffolds may consist of cell culture plates and those for 2D scaffolds may be produced easily from cut silicone sheets and glass slides. Structural materials are then polymerized, after which density modifiers may be removed by repeated washing.



SEM of 10%, 20% PEGda scaffolds created by a) DGMP and b) sequential polymerization.

In order to demonstrate the feasibility of this method for creating scaffolds in which layers have varying porosity and biological cues, we created hydrogel scaffolds from polyethylene glycol diacrylate (PEGda) and polyacrylamide. We first tested the ability of sucrose DGMP to separate chemical components by incorporating fluorescein and rhodamine B in alternating layers (Fig. 2). Fluorescence of each dye was completely separated, demonstrating that the density modifiers prevent graduation if scaffolds are polymerized immediately after layering.

To determine whether the method creates more structurally continuous interfaces between layers than sequential polymerization, two-layer (10%, 20%) PEGda scaffolds were examined by scanning electron microscopy (Fig. 3). DGMP does not produce the dense, relatively impermeable interface seen with sequential polymerization.

Because time allows layers of varying density to intermix, we sought to demonstrate that DGMP allows creation of scaffolds with graduated boundaries between layers. We varied the settling time before polymerization of two-layer (7%, 1%) polyacrylamide scaffolds and observing the change in the

lower layer's susceptibility to swelling.

Though we expect that the most biologically relevant application of DGMP would involve creation of scaffolds from biological polymers such as hyaluronic acid or collagen, we also investigated the ability of DGMP to separate biological cues and whether cells could be encapsulated prior to polymerization. C2C12 myoblasts attached only to those regions of 2D PEGda scaffolds into which RGDS peptide had been incorporated. Calcein-AM live stain confirmed that these cells survived encapsulation and polymerization into 3D PEGda scaffolds incorporating RGDS.

DGMP provides many advantages over alternative methods of creating scaffolds for the culture of complex tissue. Most importantly, this method is straightforward and economical enough to be adopted in almost any lab, as it relies on common reagents and materials. Further, it yields scaffolds with structurally uninterrupted interfaces that should allow incorporation of cells throughout, and can be adapted to create scaffolds with a variety of compositions, geometries, and porosities. Combining DGMP with additional methods would allow inclusion of additional biomimetic features, such as vascularity and release of growth or differentiation-promoting factors at specific times. DGMP thus allows fast, simple fabrication of structurally uninterrupted complex tissue scaffolds with spatially controlled bioactive chemical and mechanical cues.

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18. Design and Operation of A Bioreactor System for the Expansion of Mouse Embryonic Stem Cell-Derived Neural Stem Cells On Microcarriers

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Neural stem (NS) cells are self-renewing multipotent cells present in the developing and adult mammalian CNS. They generate the neuronal and glial cells of the developing brain and also account for the limited regenerative potential of the adult brain.

Different protocols for NS cell expansion have been developed in both floating and adherent conditions. The propagation of floating cell clusters, called “neurospheres”, is widely used for NS cell expansion *in vitro*. However, this system shows severe limitations for cell culture like heterogeneous aggregate composition, diffusion limitations of nutrients and waste accumulation in the cluster centre or preferential astroglial differentiation *in vivo*. The expansion of NS cells on adherent conditions may circumvent most of these limitations.

Different sources of NS cells are available, such as embryonic stem (ES) cells, which can give origin to NS cells by *in vitro* differentiation, the fetal brain or the adult brain. The propagation of NS cells and their further differentiation into mature neuronal phenotypes allows their potential use for treatment of neurodegenerative diseases, neural drug screening and also gene therapy. Although it is anticipated that a large number of cells will be required for those applications, the large-scale expansion and controlled differentiation of ES cell-derived NS (ESNS) cells on bioreactors has not been addressed in detail. This work aims to solve the main biological and technological hurdles that limit this application using as model a cell line of mouse ESNS.

mESNS cells are currently cultured under static conditions on tissue culture plastic. However these culture systems are not amenable for large-scale applications since they are limited by a reduced surface area/volume ratio, have a non-homogeneous nature, resulting in concentration gradients (growth factors, metabolites, pH, dissolved O_2), and their on line monitoring and control is difficult or even impossible.

The first step for establishing a mESNS cell spinner-flask culture protocol was a screening of different commercially available microcarriers, to support cell adhesion. To eliminate animal-derived products from the culture, only xeno-free microcarriers were tested, as well as a serum-free culture medium. Selected microcarriers were tested under dynamic conditions, in the spinner-flasks. Superior performance was observed with polystyrene beads coated with a recombinant protein polymer containing the RGD sequence (Pronectin F). Importantly, high cellular viability and the expression of Nestin, a marker of neural stem/progenitor cells, was retained when cells were cultured for up to 9 days on the microcarriers, in the spinner-flask, confirming that these culture conditions are not detrimental for the cells. The subsequent steps were the optimization of culture parameters. A stirring speed of 60 rpm was found to be optimal and led to an almost 35-fold increase in cell number after 6 days, with viabilities above 95%. Culture medium feeding is also a critical parameter. Glucose and lactate concentration profiles over time were determined, to avoid nutrient scarcity or metabolite accumulation. The frequency of growth factors supply was also found to be crucial. Finally, microcarrier concentration was found to have an important role as it relates with the available surface area for cell growth. Importantly, expression of Nestin, a marker of neural stem/progenitor cells, was maintained in mESNSC expanded in spinner flasks for 9 days, as well as the capacity to differentiate into neuronal and glial cells.

The study here described may constitute a starting point for the development of similar systems for larger scale bioprocesses for expansion of these and other populations of adherent NSCs, either from mouse or human origin, which may find application in clinical settings.

19. Developing an *in vitro* Model of Ectopic Calcification to Test Osteoclastic Resorptive Activity

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Ectopic calcification (EC), the inappropriate mineralization of soft tissues, occurs in disease, trauma, and aging. It is a major complication in aortic valve stenosis, orthopedic injuries and chronic kidney disease. Our lab is developing cell-based

therapies based on dimerizer-controlled osteoclast differentiation from engineered monocytes/macrophages as a treatment for EC. However, common methods to characterize osteoclastic resorption are limited by their two-dimensional nature and failure to mimic the composite matrix and mineral nature of EC. Therefore, we are developing alternative *in vitro* models to test osteoclastic resorption potential that more closely mirror the three-dimensional and mineral/matrix composition of EC. Microporous fibrin scaffolds with a pore size of 200-250 microns were generated using sphere templating technology and were mineralized with a physiological mineral solution for 48 hours, resulting in a calcium content of 34.8±5.6 µg calcium/mg dry scaffold weight. We tested the ability of macrophage-derived osteoclasts to resorb the scaffolds. Cell seeding methodology and density were optimized, with 20,000 cells/scaffold being the ideal density for osteoclast formation. Engineered osteoclasts were than compared to the parent macrophage cell line for their ability to resorb the scaffolds at various time points (days 2, 5, 8, and 11) following cell seeding. We observed a significant decrease in the weights of the scaffolds seeded with the engineered osteoclasts compared to parental macrophages by day 11. Weight loss was minimal in scaffolds that did not receive cells at day 11. When the scaffolds were examined by histology, multinucleated cells positive for tartrate –resistance acid phosphatase (TRAP) were identified in the scaffolds seeded with engineered osteoclasts, whereas no TRAP positive cells were seen in scaffolds seeded with parental macrophages. In summary, mineralized fibrin scaffolds represent an advancement over previous assay systems for measuring osteoclastic mineral resorption and matrix degradation by mimicking the three-dimensional and composite nature of EC typically observed *in vivo*.

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20. The Development of ES Cell Expansion System Using Hollow-Fiber Dialysis Membrane

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Introduction

Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are promising cell sources for regenerative medicine. Since over billions of cardiomyocytes have been reported to exist in human left ventricle, the development of the culture system to collect the enough amounts of cardiomyocytes is necessary for creating the regenerated cardiac tissue. Although several reports have shown the usefulness of the perfusion and suspension culture system of ES/iPS cells, we thought it was important to develop the system with cost- and labor-savings for the future industrialization. Here we show the usefulness of the dialysis system in the three dimensional suspension bioreactor culture of mouse ES cells.

Materials and Methods

We recently developed a robust and scalable bioprocess that allows direct embryoid body (EB) formation in a fully controlled, stirred 100 ml bioreactor with a pitched-blade-impeller following inoculation with a single cell suspension of mouse ES cells. mouse ES cells(EMG7) that express EGFP under the control of αMHC promoter, were cultivated in the suspension bioreactor system for 3 days in the GMEM supplemented with 10 % FBS. Then EBs were cultured for further 7 days with the dialysis system. Namely, EBs were cultivated in the 100 mL culture vessel and the supernatant was continuously dialyzed using hollow-fiber membrane with GMEM in the dialysis vessel.

Results and Discussion

Suspension culture with dialysis system increased the number of cells by 1.4-fold compared with that with conventional daily medium exchange methods at day 10. Furthermore FACS analysis revealed that the percentage of GFP positive cardiomyocytes was approximately 2% in both methods, suggesting that suspension bioreactor culture system with dialysis system might be effective on the cell growth. Next we examined the biochemical analysis of the medium in the dialysis system. When ES cells were cultured in the conventional medium exchange methods, pH of the culture vessel extremely decreased after day 6. On the other hand, dialysis system enabled to maintain pH around 7.2 until day 10. In consistent with the results of pH, lactate concentration in the culture vessel was remained low in the dialysis system. Finally we examined the viability of cells using Tunel staining. The number of Tunel positive cells in EBs cultured in dialysis system was markedly less than that in conventional methods. In the dialysis culture, only 10 mL FBS was used, which leads to the cost reduction up to half of the conventional methods. These findings suggest that suspension bioreactor culture with the dialysis system can be useful in terms of not only biological aspects such as cell growth, differentiation and cell viability of mouse ES cells but also cost- and labor-savings.

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21. The Development of Large Scale Continuous Perfusion Culture System for ES Cell Expansion and Cardiac Differentiation

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Introduction

Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are promising cell sources for regenerative medicine. Since over billions of cardiomyocytes have been reported to exist in human left ventricle, the development of the culture system to collect the enough amounts of cardiomyocytes is necessary for creating the regenerated cardiac tissue. We have recently developed scalable expansion culture and differentiation system for mouse ES cells. This system allows to

continuous production of cardiomyocytes, elimination of labor-intensive medium exchange and differentiation procedure by using continuously perfusion. Here we show the investigation as approaches to industrial scale production of cardiomyocytes, the usefulness of real-time viable biomass monitor for measurement of cell growth and the continuous cell concentration process.

Materials and Methods
We used a fully controlled, stirred 100mL bioreactor as preculture with pitched-blade-impeller following inoculation with a single cell suspension of mouse ES cells. Mouse ES cells (R1) that express neomycin resistant gene under the control of αMHC promoter, were cultivated for 3 days in the DMEM supplemented with 15% FBS. Then EBs were inoculated in 1L bioreactor and cultured for further 7 days with the continuous perfusion system equipped with a settling tube. In the enrichment process, G418 containing medium was continuously added for 8 days to remove the non-cardiomyocytes from a vessel. In all these liter cultivation processes, the number of viable cells was measured using the real-time biomass monitoring system (ABER Instruments, UK).

Results and Discussion
The suspension culture with continuous perfusion system was capable of increasing the number of cells upto 250 times at day 10. The capacitances measured with the real-time monitoring system were positively correlated with the number of viable cells in a vessel all over the processes, suggesting that the monitoring of capacitances might be useful for the indicator of EB growth and selection in large scale suspension culture of ES/iPS cells. After the purification step, almost all of cells were showed the spontaneous beating and expressed several cardiac proteins. These findings suggest that the large scale bioreactor system with the appropriate monitoring system might be useful to collect the target cells for the regenerative medicine.

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22. Dynamic Control Over the Mechanical Microenvironment During the Neuronal Differentiation of Mouse Embryonic Stem Cells

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Current neuronal differentiation protocols for mouse embryonic stem cells are mostly focused towards optimizing growth factor concentrations in order to maximize yields of differentiated cells. However, recent work has shown that other elements of the microenvironment, such as the mechanical microenvironment play a crucial role in regulating the survival, growth and function of many somatic cell types. However, the role of the mechanical microenvironment in regulating the attachment and neuronal differentiation of mouse embryonic stem cells has yet to be elucidated.

The scope of this study is to investigate the effect of Young's modulus on the initial attachment and early neuronal differentiation of mouse embryonic stem cells. Young's modulus was varied within an elastically tunable, gelatin-based cell culture substrate. Furthermore, the effect of Young's modulus upon attachment and differentiation of pre-differentiated mouse embryonic stem cells allows for a dynamic optimization of Young's modulus for cells as they differentiate.
Upon seeding mouse embryonic stem cells on substrates with Young's modulus varying from 2 (equal to brain and central nervous tissue) to 35 kPa (equal to demineralized bone), it was found that E14 and 46C mouse embryonic stem cells attach more strongly to substrates with lower Young's modulus compared to higher modulus substrates. Cells pre-differentiated by a specific amount were found to be less sensitive to Young's modulus in comparison with undifferentiated mouse embryonic stem cells. Pre-differentiated cells on higher moduli substrates were found to attach more strongly than undifferentiated cells on the same Young's modulus. This effect became more pronounced as the extent of pre-differentiation increased.

We will also report the development of a novel, microfluidic chip, which has been designed in order to characterize and validate the effect of Young's modulus on attachment and differentiation at a smaller scale. These smaller scale studies will also allow for experiments using smaller volumes of the expensive growth factors required for neuronal differentiation of mouse embryonic stem cells.

Optimizing Young's modulus throughout the early neuronal differentiation process will allow for a creation of more physiologically relevant *in vitro* conditions for cells, which may potentially allow for improved function *in vivo* when transplanted. Characterization of cell attachment may also be valuable in creating physiologically relevant biomaterial scaffolds for tissue engineering applications. Furthermore, it will also allow us to gain an insight into the behavior of cells when introduced to an area of the body in the diseased state, where the mechanical microenvironment may be significantly different to that of both healthy tissue and *in vitro* culture.

23. Effect of Dissolved Oxygen Tension and Medium Exchange On the in vitro Proliferation and Metabolism of Human Mesenchymal Stem Cells: A Quantitative Approach

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The potential for human mesenchymal stem cells (hMSCs) to be used for therapeutic applications has generated considerable interest therefore the ability to culture such cells*in vitro* is vital. Currently measurements taken during standard T-flask culture are usually limited to cell number, viability, confluency and those related to functionality. It is our contention that in order to form the basis for the development of any larger scale production of

hMSC's either by scale up or scale out methods, the process control factors need to be understood by the engineer.

Here we provide a quantitative analysis of the changes in concentration of the main metabolites (glucose, lactate and ammonium) with time during hMSC monolayer culture over 4 passages where a 100%, 50% and 0% growth medium exchange was performed after 72h in culture. Results from flasks held at normoxic (20% O2 /80% N_2v/v gas in the headspace, equivalent to 100% dO2 saturation in the medium with respect to air) and hypoxic conditions (2-5% O2 v/v gas in the headspace, equivalent to 10 to 25% dO2 saturation in the medium with respect to air) are compared. In general it was found that flasks where a 50% medium exchange was conducted performed better with respect to the levels of glucose consumed versus lactate produced, with the normoxic flasks yielding greater numbers of cells than the hypoxic flasks. A thorough analysis will be presented here including specific growth rates, doubling times, yield coefficients as well as cumulative time profiles for all of the metabolites measured. Such detailed data will form the basis of further larger scale studies.

24. Effects of Microencapsulated Osteoblast-Derived Growth Factors, Cell Ratio and Hydrogel Diameter On the *ex vivo* Expansion of Hematopoietic Stem/Progenitor Cells Under Hypoxia Environment

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Osteoblasts play an essential role in the construction of the hematopoietic stem/progenitor cell (HSPCs) niche, also characterized by its hypoxic environment *in vivo*. In order to successfully reconstruct *in vitro* the hematopoietic microenvironment, gelatin-alginate-chitosan microencapsulated osteoblasts and hypoxia conditions were used for the expansion of HSPCs from umbilical cord blood. The different oxygen concentrations, suitable diameter of microencapsulated beads and cell number ratio between umbilical cord blood mononuclear cells (UCB-MNCs) and rat or human osteoblasts (rOB or hOB, respectively) were determined. The microencapsulated osteoblasts and UCB-MNCs were co-cultured in serum-free medium supplemented with relatively low doses of purified recombininant human cytokines in hypoxic and normoxic incubators, respectively, whilst the self-cultured UCB-MNCs in hypoxic and/or normoxic conditions were operated as the control groups. The expansion of HSPCs was evaluated by counting the UCB-MNCs, colony-forming unit (CFU) assay and CD34+ flow cytometric analysis. After 7 days of culture, the expansion of UCB-MNCs co-cultured with microencapsulated osteoblasts with 0.5 mm diameter and cell ratio of 2:1 osteoblasts to UCB-MNCs under hypoxic conditions (5% oxygen tension) was 49.0±4.6 fold ($P<0.01$, when compared to the control group). The population percentage of CD34+ cells increased from 1.9% to 3.4% and achieved a fold-expansion of 87.6±8.3 fold. The CFU-Cs also obtained 9.8±0.8 fold expansion. In conclusion, it was demonstrated that the presence of osteoblasts had an extremely significant effect on the expansion capacity of the HSPCs under hypoxia conditions *in vitro*. This work was supported by DUT11SM09, the NSFC30700181/31170945 and The Project-sponsored by SRF for ROCS, SEM.

25. Efficient Adenoviral Transduction In Stem Cells Through Cyclic Microfluidic-Assisted Infections At Low MOI

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An efficient and safe gene transfer technique could be a powerful tool for the regulation of stem cells differentiation into lineage-committed cells and could be applied to regenerative medicine based on both ES and iPS cells. Among different technologies, adenovirus (Ad) vectors are widely used as gene delivery vehicles in both experimental studies and clinical trials. Ad vectors represent an attractive tool for gene transfer application because they are easily constructed, can be prepared in high titres, and provide gene transduction in both dividing and quiescent cells (Palmer et al., 2008, Gen Ther). Moreover, the development of protocols to culture different embryonic stem cells lines in feeder-free media (Braam et al., 2010, Methods Mol Biol) allows performing highly effective gene transductions experiments.

Nevertheless, the use of high viral doses to achieve an efficient expression of target genes could induce toxic effects on host cells, as reported in many studies (Liu et al., 2003, Gene Ther). It is therefore essential to obtain a high efficiency of infection by using low viral doses, in order to avoid any innate response of host cells to adenoviruses. Miniaturized devices, such as microfluidic platforms, represent very useful tools to enhance virus mass transfer from viral suspension bulk to the cellular layer. In fact, they are ideal in controlling very small volumes of medium over the cell culture by tuning the microfluidic geometry and the frequency of sequential virus delivery.

In this perspective, we aimed to develop a microfluidic system, in order to enhance the efficiency of infection in target cells at low doses of viruses and maintain a high expression of transduced proteins. To achieve this goal, we designed a microfluidic platform with 10 independent microfluidic channels, in which stem cells are cultured for long term and cyclically infected at very low MOIs, leading to an elevated percentage of spatially homogeneous infected cells. Moreover, in these experiments, toxic effects are avoided and sustained expression of transduced proteins over time are obtained. In order to assist the design of experiments, we develop a mathematical model, based on the stochasticity of viral infection process. This model fairly predict the number and the distribution of infected cells as a function of number of infecting viruses per cells.

The model was experimentally validated by infecting human fibroblasts with an Ad-EGFP virus vectors at MOI 1, 5, 10 and 20. In general, theoretical profiles appear in very good agreement with the fluorescence intensity distribution of EGFP-expressing cells. However, at higher MOI, experimental efficiency of infection is not consistent with the modeling outcomes; agreement between experimental results and theoretical predictions are only possible by assuming cell death, as consequence of a high simultaneous viral infection. On the other hand, cyclic infections at low MOI results in fully predictable virus infection efficiency and fluorescence intensity distribution of EGFP-expressing cells by stochastic simula-

tion. For instance, repeated incubation of the cell culture every 12h for 2 days at MOI 5 leads to an 80% of EGFP-positive cells after 60h from starting experiment, whereas only 60% EGFP-positive cells were detected by infecting the same cell culture only one time at the same total MOI. Consistent results are obtained for human hIPSc, meaning that the developed technology could be very worthwhile to obtain an efficient protein expression in target stem cells.

26. Electrically Conductive, Biocompatible Composite Containing Carbon Nanobrushes for Applications in Neuroregeneration

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Over 90,000 people suffer from injuries and damages to the nervous system every year [1]. The central nervous system (CNS), which consists of the brain and the spinal cord, does not have the intrinsic ability to regenerate and repair damaged neurons, and there are currently no known treatments to achieve nerve function recovery after CNS injuries [2]. In the event of neurological damage, nerve tissue regeneration relies on the re-extension of axons, which conduct electrical impulses away from the nerve cell body. Cho and Borgens have used SEM and immunofluorescent imagery to show strong evidence that electrical stimulation can significantly influence the growth and morphological features of cells used for neuronal differentiation [3].

The aim of this project is to design an electrically conductive composite containing carbon nanobrushes (CNBs) for applications in nerve cell regeneration. The composite will be prepared by suspending carbon nanobrushes in a biocompatible hydrogel such as Pluronic F127 poloxamer gel, a reverse phase-change mixture of variable chain length polyethylene glycol (PEG) that is hydrophilic and non-ionic. The end-design will serve as a standard matrix for experiments involving stem cell and nerve cell growth in electrically conductive environments. This biocompatible composite is expected to be very important for enhancing the growth, differentiation, and branching of neurons *in vitro* in an electrically driven way. Furthermore, the project will involve the formulation of a method to measure the electrical properties of the composite.

Hydrogels can serve as important components in the extracellular matrix for cells, because they provide favorable surface area for cell adhesion and structural support in the form of three-dimensional encapsulating scaffolds [4]. Kawaguchi showed that an alginate gel containing carbon nanotubes provided a suitable scaffold for tissue engineering with no cytotoxicity [5]. Carbon nanotube content is known to have conductive properties because of its symmetry and the unique electronic structure of graphene [3]; therefore, hydrogel composites with imbedded carbon nanobrushes are likely to inherit conductive properties as well [6], making them suitable for experiments involving nerve cell regeneration.

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27. Endogenous Stem Cell Mobilization and Homing for Bone Regeneration Modulated by Sphingosine-1-Phosphate (S1P) Receptors

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We have previously shown that coating allografts with FTY720, a S1P3 agonist, increases the rate of critical size defect healing by enhancing homing of host-derived CXCR4+ stem/progenitor cells such as mesenchymal stem cells (MSCs). In this study we show that pharmacological inhibition of S1P3 via VPC01091 significantly increases mobilization of BMSCs into peripheral blood resulting in accelerated bone repair in rat cranial defects. Additionally, MSCs pre-treated with FTY720 exhibit increased migration towards SDF-1, a CXCR4+ ligand and critical component of the bone marrow niche. These findings advocate the significant role of S1P3 in stem cell chemotaxis. We show that treating animals with both FTY720 coated allografts locally, and VPC01091 systematically is beneficial if controlled temporally. We propose that S1P3 receptor antagonists aids in the mobilization of MSCs, while agonists of the same receptor are critical for stem cell recruitment. Thus, suggesting the presence of a push-pull mechanism that is dictated by S1P receptor specific small molecules.

5mm cranial defects were made in 36 nine weeks old Sprague Daley rats, which were divided into 4 groups (n=9). The rats were treated with a systemic dose of 1 mpk VPC01091, FTY720 coated semi-circular allograft, FTY720 coated semi-circular allograft + a systemic dose of 1 mpk VPC01091 or left untreated. VPC01091 was given the day after surgery and 3 weeks post surgery. Hemavet (Drew Scientifics) was used to measure the concentrations of blood cells at days 0, week 1 and week 2 (n=6) (data not shown). The amount of bone regeneration was measured bi-weekly with microCT imaging (n=3-9). Flow cytometry was performed according to standard procedures on the tissue harvested from the defect sites at week 3 (n= 3), and from peripheral blood at week 6 (n=3). Monoclonal antibodies (Invitrogen, Abcam) for rat CD45, CD11b, CD54, CD90 were used in both cases. Mason's Trichrome and H&E staining were done for done for all groups (n=3).

Treatment with systemic VPC01091 resulted in substantial bi-weekly increase in bone regeneration compared to the empty defect controls. This group also showed an increase in the % of CD54 and CD90 positive cells (rats MSC markers) in the defect region at week 3 and in the blood at week 6. Animals treated with FTY720 allografts showed a temporal response to VPC01091. Initially, they showed lesser bone growth compared to just FTY720 treatment, but the trend reversed after week 4.

These results indicate that a systemic treatment with VPC01091 will significantly accelerate bone regeneration in the absence of any local implant. However, the effectiveness of locally released FTY720 to promote healing requires recruitment of BMSCs via S1P3, suggesting that the time of systemic delivery of a S1P3 anatgonist is crucial for the body to engage in this push-pull mechanism of endogenous stem cells. This manifests in the fact that the rate of increase in bone volume at later time points is the highest for the group treated with both FTY720 allograft and VPC01091. The presence of an increased number of MSCs both in the blood, and defect region tissue denotes that the cells required for bone healing are being mobilized into the blood, and recruited to the defect site as late as 6 weeks after injury. Thus, this study shows that the rate of bone growth in large defects can be controlled by a combination of S1P receptor specific small molecules in a time dependent manner. The recruitment of CXCR4+ stem/progenitor cells and enhancement of bone defect healing via neovascularization and osseous tissue in-growth can be achieved through selective targeting and activation of S1P receptors.

We propose the systemic use of a S1P3 receptor antagonist, VPC01091, to mobilize endogenous stem cells in order to increase bone regeneration. Such endogenous stem cell therapy can be used to enhance bone regeneration in instances when there is substantial soft tissue damage and/or exogenous stem cell transplant is not feasible. Endogenous stem cell therapies have been used in various other ailments like cardiovascular infarctions and can prove to be as effective in bone healing.

28. Engineered Neural Tissue with Columns of Aligned Schwann Cell-Like Cells From Differentiated Adipose-Derived Stem Cells Can Support and Guide Neuronal Growth

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There is a clear unmet clinical need in the repair of peripheral nerves where damage has resulted in a long gap. The current clinical 'gold standard' is to use an autograft. However, this causes donor site morbidity, has limited availability and often leads to poor functional outcome. Another approach is to use biomaterials conduits. However, current clinically approved hollow nerve guidance conduits lack the trophic support and guidance cues provided by Schwann cells in an autograft, thus limiting their use to treating a relatively short lesion only. We have investigated a third option: Tissue-engineered cellular bridging devices for surgical implantation into peripheral nerve injury sites could provide an attractive alternative to the autograft. In order to direct neuronal growth across a lesion to shorten the delay of reinnervation and improve functional recovery after injury, such a device should mimic the beneficial features of a nerve autograft including the cell-level guidance and trophic support provided by the Schwann cells. The ability of Schwann cells to enhance axon migration and secrete factors that further increase regeneration has been well established. However, the use of autologous Schwann cells within an engineered bridging device for long gap repair has a number of disadvantages, including the sacrifice of host nerve tissue for their isolation, and lengthy expansion times in vitro. An alternative to autologous Schwann cells that avoids the challenges associated with allogeneic cells is to use stem cells from a patient's own adipose tissue. Adipose derived stem cells (ADSC) that have been differentiated towards a Schwann cell-like phenotype in vitro (dADSC) have the potential to provide the trophic support and pro-regenerative behaviour elicited by the Schwann cells in an autograft. Here we show that they can form the basis of a guidance substrate in a peripheral nerve repair device. This study reports the development of a living replacement tissue which mimics key cellular and extracellular features of the autograft endoneurium, using therapeutically relevant dADSC to form an engineered neural tissue (ENT). The ENT is made from a cellular collagen gel that is tethered at each end to permit the dADSC to self-align and form columns. This anisotropic cellular hydrogel is subjected to a stabilisation process that removes some interstitial fluid to produce a robust biomaterial with the correct tissue architecture. Cell death assays, immunostaining and confocal microscopy showed that dADSCs can be successfully used to form ENT and maintain their phenotype - dADSCs in collagen gels survive and maintain their alignment following the stabilisation process to form sheets of aligned cellular collagen biomaterial.in vitro testing revealed that dissociated dorsal root ganglion neurons seeded onto the surface of flat sheets of ENT extended neurites that were guided by the orientation of the aligned dADSCs. Sheets of ENT containing rat dADSC were integrated into a repair device by rolling ENT into aligned tissue columns. These constructs were then packed together within a clinically approved tube, NeuraWrapTM and tested *in vivo* using a rat sciatic nerve repair model to assess the potential of the device to offer an alternative to the nerve autograft.

29. Engineering Cellular Homing and Migration to Enhance Immune and Stem Cell-Based Therapeutics

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A critical obstacle to the effectiveness of cellular therapies is the inadequate long-term homing, retention, survival, and integration of transplanted regenerative cells into damaged tissues. Successful future therapeutic application will likely depend on improvements in these areas.
By utilizing a synthetic biology approach to "program" useful func-

tionalities into cells through genetic modification, we seek to enhance the homing of cells to sites of disease. Using immune cells as a test-bed for our cellular engineering efforts, we have discovered that an engineered G-Protein Coupled Receptor (GPCR) activated solely by a biologically inert small molecule metabolite clozapine N-oxide is sufficient to redirect the migration of neutrophils and T lymphocytes in cell-based assays as well as in a mouse model. Genetically modified T lymphocytes have been used in human studies for the last two decades, making them an ideal platform for the study of cell migration and homing.

Further, we are extending our efforts to study a class of pathologies in which defects in cellular migration are strongly implicated: neurocristopathies, which are diseases involving defects in the development of tissues containing cells derived from the neural crest cell lineage. We have generated neural crest stem cells from patient-specific induced pluripotent stem cells and are working to both study defects in cell migration in cell-based assays as well as to investigate the effects of reconstituting migration in these cells through an engineered GPCR. We envision that our work will help us better understand the cellular basis of disease in neurocristopathies and aid in therapeutic efforts to treat such pathologies through gene therapy and cell-based treatments.

30. Engineering of Human Bone Tissue From Pluripotent Stem Cells In Perfusion Bioreactors

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Bone tissue engineering could provide an unlimited supply of functional viable bone grafts, needed to repair large bone defects resulting from trauma, congenital malformations and surgical resections. Human embryonic stem cells (ESC), and the embryonic-like induced pluripotent stem cells represent a promising cell source for this goal, as they can grow indefinitely, providing unlimited numbers of tissue repair cells, and give rise to any cell type in the body. In prior studies, limited formation of bone tissue was observed, accompanied by the development of teratomas. The goal of our study was to demonstrate the feasibility of engineering in a controllable manner fully viable, ~0.5 cm large compact bone constructs, and to evaluate their phenotypic stability and safety in a subcutaneous implantation model.

We developed a stepwise engineering protocol, where ESC were first induced into progenitors expressing mesenchymal surface markers and in vitro differentiation potential into osteogenic, chondrogenic and adipogenic lineages. Cultivation of ESC-mesenchymal progenitors in osteoconductive scaffolds in perfusion bioreactors for 5 weeks resulted in compact tissue constructs with significantly higher cell numbers, alkaline phosphatase activity and osteopontin release into culture medium compared to static cultures. Engineered tissue contained dense, homogenously distributed bone matrix, as observed by positive immunohistochemical staining of osteopontin, bone sialoprotein and osteocalcin, positive Goldner's Trichrome staining of osteoids, and increased mineralized matrix content, detected by micro-CT imaging.

Engineered bone tissue matured during the 8 weeks of subcutaneous implantation, resulting in denser bone matrix compared to scaffolds seeded with ESC-mesenchymal progenitors prior to implantation. Further maturation of the mineralized matrix was detected by micro-CT imaging. There was no evidence of teratoma formation, which was found in control group of scaffolds seeded with ESC. In addition, engineered bone constructs contained microvasculature spanning the interior regions of the scaffolds, and osteoclasts in the outer regions, suggesting initiation of tissue remodeling.

Our results demonstrate ESC-mesenchymal progenitors can be induced to form compact, homogenous and phenotypically stable bone-like tissue in controllable fashion, by cultivation on three-dimensional osteoconductive scaffolds in bioreactors with interstitial flow of culture medium. We propose that engineering bone-like tissue from human pluripotent cells can help advance fundamental study of osteogenesis, as well as translation into regenerative medicine applications.

31. Engineering the Microenvironment of the Adult Brain to Direct Neural Stem Cell Fate

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Neurogenesis in the adult hippocampus involves activation of quiescent neural stem cells (NSCs) to yield transiently amplifying NSCs that differentiate into neurons. These processes are tightly regulated by microenvironmental cues; however, few endogenous factors are known to regulate neuronal differentiation, and the role of juxtacrine (i.e. cell-cell contact-dependent) signaling has not been broadly investigated. We show that administration of ephrin-B2 increases neurogenesis in vitro and *in vivo*. Within the niche, hippocampal astrocytes express ephrin-B2, and RNAi-induced knockdown of this transmembrane ligand in cultured hippocampal astrocytes and within the hippocampus decreases neuronal differentiation of co-cultured and resident NSCs, respectively.

Additionally, ephrin-B2 signaling is transduced by NSC EphB4 receptors, activates β-catenin in vitro and *in vivo* independent of Wnt signaling, and upregulates proneural transcription factors. Ephrin-B2+ astrocytes thus promote neuronal differentiation of EphB4+ adult NSCs through ephrin-B2 juxtacrine signaling, findings that advance our understanding of adult neurogenesis and may have future regenerative medicine implications.

32. Evaluation of Continuous, Scalable Concentration and Wash Systems for Cellular Therapies

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The separation of cells from process fluids for the purposes of

concentration and washing the cells can be readily achieved at small scale by batch centrifugation, which separates using principles of density-based sedimentation. This process is labor intensive, open to the environment, and not scalable. The goal of this project was to evaluate cell concentration and washing technologies that offer scalable and disposable closed system alternatives to batch centrifugation.

Two varieties of unit operations, continuous centrifugation (CFC) and tangential flow filtration (TFF), were selected for evaluation. Cell recovery, cell health, and process fit were significant metrics in this project. Design space assessments were performed for flux, pore size, feed flow rate and lumen diameter in TFF. Continuous centrifugation design space of process fluid density and flow rate were characterized.

Results demonstrated that both continuous cell wash systems yielded healthy cells with stable critical quality attributes and recoveries over 85%.

33. Expansion of Human Embryonic Stem Cells On Coating-Free Microcarriers In Serum Free Conditions

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The promise of human embryonic stem cells (hESCs) in the field of regenerative medicine is widely recognized due to its proliferative ability and potential to differentiate into cells of different lineages. Over the recent years, hESCs culture systems have been improved and developed from initial protocols using feeder cells, to the use of feeder-free systems and currently, fully defined serum-free media in either 2D colony or microcarrier cultures. However, culturing of hESCs on tissue culture flasks or microcarriers requires the coating of extracellular matrices such as Matrigel™ (BD), obstructing the process of establishing a fully-defined, robust and animal component free scalable platform for hESCs expansion.

This study reports that the addition of a small molecule, ROCK inhibitor Y27632, enables long-term culturing (> 10 weeks) of hESCs on cellulose based rod shape microcarriers DE53 without the need for any extracellular matrix. ROCK inhibitor-treated hESCs microcarrier cultured resulted in comparable cell yields (1-1.2 million cells/ml) and pluripotency (>80 % TRA-1-60 positive cells) to those on Matrigel coated microcarriers in 2 hESC lines, HES-3 and H7. In spinner flask cultures, both Matrigel-coated and ROCK inhibitor supplemented cultures achieved cell densities of 2-2.5 million cells/ml and >80% of cells expressing TRA-1-60. Cells expanded on coating-free microcarriers were further capable of differentiation to three lineages both in vitro by generating embryoid bodies and *in vivo* by teratoma formation in SCID mice. Investigation of the mechanism of action shows that the ROCK inhibitor seems to act through the Rho-ROCK-Myosin signaling pathway. This was further confirmed when the addition of myosin inhibitor Blebbistatin, which acts downstream of ROCK inhibitor in the pathway, also enabled the cultivation of hESCs on uncoated microcarriers.

It is hypothesized that ROCK inhibitor reduced the dissociation-induced apoptosis and reorganized the actin cytoskeleton network allowing hESCs to attach, adapt and grow on uncoated microcarriers.

34. Expansion of Human Mesenchymal Stem Cells On Microcarriers Under Different Dissolved Oxygen Tensions

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The use of human mesenchymal stem cells (hMSCs) for therapeutic applications has generated significant interest in their in vitro culture. However key issues need to be addressed before hMSCs are utilised routinely for regenerative medicine applications. Amongst them is the need to reproducibly obtain large numbers of well-characterised cells, which can only be achieved by identifying appropriate large-scale culture methods for stem cells and subsequently optimizing them. This work addresses this challenge using human bone marrow-derived mesenchymal stem cells (hbmMSCs), concentrating on the processing conditions required using microcarriers in 100 ml spinner flask culture.

The work presented begins with the culture of hbmMSCs and the screening of commercially available and in-house produced microcarriers. Microcarriers which demonstrated high levels of attachment and proliferation were then taken forward for hbmMSC culture in agitated 100 ml spinner flasks where viable cell number and daily nutrient/metabolite concentrations were measured. Static control spinner flasks were run in parallel with the agitated flasks (30 rpm after an initial 24 hr agitation delay) at normoxic (20% O2 / 80% N_2 v/v gas in the headspace, equivalent to 100% dO2 saturation in the medium with respect to air) and hypoxic conditions (2-5% O2 v/v gas in the headspace, equivalent to 10 to 25% dO2 saturation in the medium with respect to air). The agitated conditions performed better with respect to glucose consumed versus lactate produced, with the higher dO2 giving a higher cell concentration. Moreover, cell yield was comparable to T-flask monolayer culture of an equivalent surface area (500 cm2), with approximately 20 x 106 cells obtained on the microcarriers which demonstrated the greatest cell proliferation. It was also found that neutrally charged microcarriers yielded a greater cell number. Given the advantage of scale for microcarrier culture, the results are promising.

This is the first time that microcarrier culture at different dO2 has been directly compared with monolayer culture. This information will now provide a platform for the scale-up to a 5 L bioreactor.

35. Exposure of Mesenchymal Stem Cells to Electric Current Induces Osteodifferentiation

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Progress in developing clinically-relevant biomedical applications (such as regeneration and repair of damaged tissues) using pluripotent stem cells has been slow because the conditions to generate either the required numbers of undifferentiated cells or lineage-specific cells remain, at best, partially understood. To date, biochemical compounds (such as select growth factors, etc.) have been utilized to promote differentiation of pluripotent stem cells into specific cell phenotypes. In the case of bone marrow-derived mesenchymal stem cells or MSCs (which have the potential to differentiate into either osteoblasts, chondrocytes, or adipocytes), bone morphogenetic proteins have been used to induce differentiation into osteoblasts, the bone forming cells. In contrast, the effects of biophysical stimuli on such outcomes remain unknown.

The present study addressed the need for novel strategies that reliably induce MSC differentiation into specific cell types and investigated the effects of electric current, a biophysical stimulus alone, i.e., in the absence of supplemented exogenous growth factors. Motivation for the study was provided by the physiological milieu: bone and its constituent cells exist, and function, in an environment composed of biochemical as well as by biophysical (specifically, mechanical and electrical) stimuli. For this purpose, a custom-made laboratory set-up was used to expose adult human mesenchymal stem cells (passage 3-5) to alternating electric current (either 10 or 40 μA, 10 Hz frequency, sinusoidal waveform), for 6 hours daily for up to 14 consecutive days. Cells were cultured on electrically conductive materials, i.e., either flat, indium-tin-oxide-coated glass (pre-coated with fibronectin) or dispersed within collagen (rat tail Type I) cylindrical hydrogel scaffolds in the absence of supplemented exogenous growth factors. Viability and cell proliferation were determined using standard laboratory techniques while cell differentiation was determined using molecular-level techniques. Modulation of mRNA and protein levels corresponding to genes known to be expressed during osteoblast (early: specifically, TAZ, Runx-2, and osterix; and late: specifically, osteopontin and osteocalcin), chondrocyte (type II collagen), and adipocyte (Fatty Acid Binding Protein-4) differentiation were monitored. Controls were cells cultured in parallel under similar conditions but not exposed to alternating electric current.

All adult human mesenchymal stem cells cultured on the substrates tested in the present study remained viable when exposed to the aforementioned electric current regime. Under these conditions, proliferation of cells cultured on flat, indium-tin-oxide glass, and within collagen hydrogel constructs were similar to that of the respective controls for the duration of the study. Compared to results obtained from the respective controls, genes indicative of osteoblast, but not of chondrocyte or adipocyte, differentiation were expressed earlier and at higher levels when the cells were exposed to alternating electric current. In summary, these results provided the first evidence that exposure to alternating electric current promotes exclusive differentiation of mesenchymal stem cells into the osteoblastic lineage.

The system tested in the present study is a potentially scalable one and presents a valuable resource for the production of osteoblasts for various bone-related biomedical applications both *in vivo* and *in vitro*. Differentiation of mesenchymal stem cells under the conditions established in the present study can provide critically needed cell supplies for cell-based assays and/or therapies needed for regenerating and repairing damaged tissues.

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36. The Extracellular Matrix Is a Novel Attribute of Endothelial Progenitors and of Hypoxic Mature Endothelial Cells

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Introduction: Endothelial cells (ECs) form the dynamic inner lining of blood vessels; this interior layer plays a pivotal role in providing necessary nutrients, facilitating gas exchange, and maintaining tissue homeostasis, among many other functionalities. Stem cells aid the growth and repair of blood vessels; specifically, endothelial progenitor cells (EPCs) that circulate in the bloodstream can be endogenously triggered to hone to sites of ischemia or form vasculatures. Because of their regenerative capacity, EPCs have been widely used in the development of vascular therapeutics.

Concentrically surrounding the endothelial layer is its extracellular matrix (ECM), which not only provides the structural framework to support cellular functions but also influences cell behavior through integrin-mediated activation and downstream signaling events. The vasculature is an intricate and complicated network requiring a multifaceted cascade of events to occur for angiogenesis, during which cellular interactions with the ECM is vital.

Hypoxia contributes to the complex interplay between vascular cells and their ECM. It critically regulates blood vessel growth in ischemic, injured, or cancerous tissue by increasing the expression of numerous genes involved in vessel formation and maturation. Hypoxia-driven angiogenesis is vital for ECM formation in tissue repair. As such, it has been an emerging focus of vascular tissue engineering.

Engineering functional replacements for or repairing damage within the vasculature remains a critical aim in vascular tissue engineering. The ECM is an important part of this microenvironment and has vast applications in engineering vasculature. Thus, close examination of ECM properties is vital to the development of vascular therapeutics.

Aims: In the present study, we sought to distinguish ECM characteristics of human arterial, venous, and progenitor ECs to elucidate variations in ECM composition and response to physiological and biochemical factors. We studied human umbilical artery ECs (HUAECs), human umbilical vein ECs (HUVECs), and endothelial progenitor cells (EPCs). We postulate that these studies would provide the necessary framework for understanding vasculature ECM in development and

disease as well as progressing vascular therapeutics.

Results: Our analyses revealed that only EPCs were able to produce copious amounts of ECM proteins (i.e. collagen IV, laminin, and fibronectin) in atmospheric conditions. After 4 days, ECM proteins collagen IV, laminin, and fibronectin, were visible extracellularly in an intricate network. These structures were still observed after 7 days of culture. HUAECs and HUVECs deposited little to no ECM after 4 and 7 days of culture. Evidence of ECM proteins at these time points were constrained to within the cells, and no intricate network resembling that from EPCs was observed, even after 7 days. In order to confirm extracellular deposition of ECM proteins by EPCs, cultures were decellularized and immunofluorescent staining images of decellularized cultures confirmed our aforementioned findings. An intricate network of fibronectin and collagen IV was evident as deposited matrix.

To elucidate whether EPC-derived ECM was regulated by biochemical factors, we examined the effect of TGF inhibition and Rho kinase inhibition. When TGF signalling pathways were inhibited, the collagen IV and fibronectin network formations were completely abrogated, though both ECM proteins were deposited by EPCs. When the cytoskeletons of EPCs were disrupted via Rho kinase inhibition, much less deposited fibronectin was observed. Collagen IV was still deposited, however without any distinct structure. These studies indicate that EPC-derived ECM is regulated via TGF signaling and cytoskeletal formations.

With the prominence of hypoxia in ischemic and injured tissues, we next examined if ECM deposition is affected by low oxygen environments. Remarkably, when exposed to hypoxic conditions, all three cell types deposited striking amounts of ECM proteins as indicated by immunofluorescence microscopy. Both HUAECs and HUVECs significantly increased collagen IV and fibronectin production. Hypoxic conditions did not seem to increase the production of these ECM proteins from EPCs, which already produced abundant ECM in atmospheric conditions. Inhibition studies were further performed to elucidate the hypoxic signaling pathways involved.

Conclusions: To the best of our knowledge, our work is the first to suggest that EPCs contribute not only to the cellular inner lining of blood vessels but also to blood vessel structure via abundant ECM deposition. We found that EPCs produce copious amounts of ECM undetected in HUAECs or HUVECs under atmospheric conditions. Consequently, this work suggests that EPCs should be considered for the engineering of blood vessels via their deposited ECM, which could provide autologous alternatives to traditional approaches.

This work also sheds light on the effect of hypoxia on vasculature ECM. Under hypoxic conditions, mature ECs resemble EPCs with respect to collagen IV and fibronectin production. Hypoxia is prevalent in injury and tumors, implying that continued studies on deposited ECM of vascular cells may provide much insight into injury recovery or tumor progression. Altogether, these studies can be used as a platform to examine the role of vascular cell-derived ECM in development and disease.

37. Feeder-Free mRNA Reprogramming Methods for the Derivation of Clinically Relevant Human IPS Cell Lines

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To date, the translation of induced pluripotent stem (iPS) cell technology from research labs to regenerative medicine and drug screening applications has been limited by the inability to derive clinically relevant human iPS cell lines. Existing methods are either inefficient and cannot be considered for widespread application or yield iPS cells that contain genomic integrations or viral contaminants resulting from the derivation methods. Recently, advances in mRNA reprogramming methods have been used to efficiently generate integration-free iPS lines in a wide variety of patient fibroblasts. Here we present data demonstrating the derivation of mRNA iPS cell lines from human fibroblasts in a feeder-free culture environment. This novel protocol eliminates the biological variability associated with feeder based reprogramming methods by pairing a defined, xeno-free cell culture medium with cell culture attachment substrates. In addition, the protocol incorporates a novel RNA transfection reagent that allows for both a reduction in the amount of RNA required on a daily basis and the number of transfections ultimately required for iPS cell colony establishment. Combined, these developments result in a human iPS cell line derivation protocol that is simple, highly efficient, as well as integration and feeder-free.

38. Fluid Shear Stress Pre-conditioning Enhances Embryonic Stem Cell Endothelial Differentiation under Low Oxygen Conditions

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Endothelial differentiation and vascularization during embryonic development are tightly regulated by environmental cues such as fluid shear stress and oxygen. Oxygen is a critical regulator of endothelial and vascular development [1, 2]. Oxygen levels within the embryo range from 1-3% [3, 4] indicating that embryogenesis occurs in a lower oxygen environment than what is typically used for *in vitro* culture. During development, oxygen gradients develop within the embryo and are responsible for patterning and formation of the cardiovascular and vascular system [2, 5]. Fluid shear stress has been explored as a method for directing stem cell endothelial differentiation. Embryonic stem cells (ESCs) have the ability to respond to fluid shear stress by changes in gene expression and cell proliferation. Researchers have demonstrated that fluid shear stress induces ESCs to differentiate towards vascular cells [6, 7], including endothelial cells [8]. Many studies have examined using oxygen and fluid shear stress independently to direct ESC differentiation; however few have examined them in combination. Therefore, the following studies investigated how fluid shear stress and varying oxygen levels affect ESC endothelial differentiation.

D3 mouse ESCs were seeded on collagen IV coated glass slides at a density of 20,000 cells/cm2 and cultured for 48hrs to allow the ESCs to form a confluent monolayer. Next, the ESCs were cultured statically or under shear conditions at 5 dynes/cm2 shear for 48hrs in a parallel plate flow chamber. Following fluid shear stress preconditioning, ESCs were further cultured as EBs. Embryoid bodies (EBs) were formed from single-cell suspension of 2x106 cells in a 100mm2 petri dishes on a rotary orbital shaker at 40RPM. EBs formed from pre-conditioned ESCs were cultured for 7 days under normoxic (21% O_2) or under physiological (3% O_2) conditions. Phase images were taken at days 2, 4, and 7 days. Preconditioned day 7 embryoid bodies were assessed for protein expression of vascular endothelial cadherin (VE-Cadherin) and platelet endothelial cell adhesion molecule (PECAM) using immunostaining and confirmed using immunofluorescence. To assess gene expression of preconditioned ESCs, qRT-PCR was performed following shear preconditioning and on days 2, 4, 7, and 10 during embryoid body culture. Expression of endothelial marker genes Flk-1, Flt-1, VE-Cadherin, and PECAM were assessed. Additionally, protein and gene expression of hypoxia inducible factor-A (HIF1) and angiogenic factor VEGF were assessed through qRT-PCR and western blots.

Shear pre-conditioned EBs cultured at 3% O_2 developed large opaque centrally located regions, while shear pre-conditioned EBs cultured at 21% O_2 developed smaller such regions. Although noticeable differences in EB morphology were observed, no apparent differences in cell viability were observed in EBs cultured at 21% O_2 and 3% O_2. Following 4 days of culture under 3% O2 EBs expressed similar levels of Flk-1, Flt-1,VE-cadherin, and PECAM compared to EBs cultured under 21% O_2. However, by day 7 EBs cultured under 3% O_2 expressed significantly ($p<0.05$) higher levels of endothelial marker genes compared to EBs cultured under 21% O_2. Additionally, at day 7, VE-cadherin positive cells in shear pre-conditioned EBs cultured under 3% O_2 organized into primitive networks which extended in all directions within the EB. While, VE-cadherin positive cells clustered at the center of shear pre-conditioned EBs cultured under 21% O_2. EBs cultured under 3% O2 which were formed from shear pre-conditioned ESCs produced significantly ($p<0.05$) higher levels of VEGF compared to EBs cultured under 3% O_2 which were formed from ESCs not pre-conditioned with fluid shear at day 7. Furthermore, EBs cultured under 3% O_2 expressed higher levels of VEGF genecompared to EBs cultured under 21% O_2. Following 7 days of exposure to 3% oxygen shear pre-conditioned EBs expressed higher levels of HIF1a protein compared to EBs exposed to 3% oxygen formed from ESCs cultured under static conditions.

Overall, fluid shear pre-conditioned ESCs exposed to physiological levels of oxygen developed endothelial-like cell networks, expressed higher levels of HIF1 , and produced increased concentrations of VEGF. These studies demonstrate that fluid shear stress pre-conditioning of ESCs modulates EB endothelial differentiation, vasculogenesis, and angiogenic factor production under different oxygen conditions. Culturing EBs formed from shear pre-conditioned ESCs under low oxygen conditions maybe a novel method to produce pre-vascularized cellular based tissue engineered products for regenerative medicine applications.

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39. Human Embryonic Stem Cell-Derived Cardiomyocytes Migrate In Response to Fibronectin and Wnt5a

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An improved understanding of the factors that regulate the migratory behavior of human embryonic stem cell-derived cardiomyocytes (hESC-CMs) would provide new insights into human heart development and perhaps suggest new strategies to improve their electromechanical integration following intracardiac transplantation. Thus far, nothing has been reported about the molecules that control hESC-CM migration, but we hypothesized that these cells would migrate in response to extracellular matrix and soluble signaling molecules known to be important in heart morphogenesis. To test this hypothesis, we screened candidate factors for effects on hESC-CM motility using the transwell assay, followed by validation via live cell imaging and/or gap-closure assay. In our initial screens with various ECM substrates, we identified fibronectin to be pro-migratory, with hESC-CMs on fibronectin showing ~10-fold greater migration than those on laminin. Fibronectin also evoked a haptotactic response from hESC-CMs, as evidenced by transwell experiments and assayed migration via live imaging of hESC-CMs on two-dimensional gradients of fibronectin generated by diffusive printing techniques. Studies with neutralizing antibodies indicated that these effects were mediated by integrin alpha-5. Transwell screens of soluble molecules identified the non-canonical Wnt, Wnt5a, as a second pro-migratory factor, which elicited a ~two-fold increase in migration over controls. This effect was confirmed using the gap closure assay, in which Wnt5a-treated hESC-CMs covered nearly twice as much surface area as untreated control cells. (There was no difference between the latter groups in terms of cell number or cell size.) In summary, we have demonstrated that hESC-CMs migrate in response to fibronectin and Wnt5a *in vitro*, and we speculate that by manipulating these signals *in vivo*, we can enhance the integration of hESC-CM grafts in infarcted hearts.

40. Human Pluripotent Stem Cell-Derived Cardiac Micro-Tissue Particles for Myocardial Infarct Repair

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We have designed scaffold-free cellular tissue particles for injection into the body as a regenerative medicine therapy following myocardial infarction. Human pluripotent stem cell-derived cardiomyocytes form tissue particles in PDMS microwells, enabling scalable production. Precise control over cell composition and numbers allows the generation of heterogeneous, spherical tissue particles with precise diameter, which we call "micro-tissue particles". Micro-tissue particles form overnight and have been cultured up to 10 days. Two thousand input cells per particle yields a spherical diameter of approximately 250 µm, and particles tolerate heat shock the day prior to implantation with minimal swelling. Cell composition is varied upon formation to create cardiomyocyte-only, vascular (endothelial cells + mesenchymal cells, 2:1 ratio), or myocardial (cardiomyocytes + endothelial cells + mesenchymal cells) micro-tissue particles. A myocardial infarction is induced in athymic rat hearts by occluding the LAD coronary artery for 60 minutes followed by reperfusion. Four days after infarction, micro-tissue particles are injected intramyocardially with a 24 gauge needle. Immediately after injection, graft size was 3% of LV area by histology with 5 x 106 total cells (~3300 micro-tissue particles of 1500 cells each), while scar was 17% of LV area in one animal. After 2 weeks *in vivo*, human endothelial cells in micro-tissue particles formed lumens by human CD31 staining and engrafted human cardiomyocytes developed striations by beta-myosin heavy chain staining. Major advantages of micro-tissue particles for cell-based therapeutics are (1) cells maintain cell-cell and cell-matrix contacts during implantation to improve cell survival and graft size, (2) particle delivery is minimally invasive and can be accomplished via catheter and needle, and (3) omitting exogenous scaffold materials minimizes the host inflammatory response to the implant. Thus, we have developed a novel therapeutic that combines tissue engineering with needle delivery of cells and describe the use of micro-tissue particles in the complex setting of heart repair after myocardial infarction as both a neo-vascular therapy and a cardiac regeneration therapy using human pluripotent stem cell-derived cardiomyocytes.

41. Image Analysis for Evaluation of Growth and Differentiation of Embryonic Stem Cells

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Ultimately tissue engineering aims to restore function to tissues identified as the underlying cause of illness. One of the most important building blocks used in tissue engineering are living cells. Embryonic stem cells (ESCs) are rapidly emerging as a promising cell source due to their unique characteristics-namely their ability to readily proliferate in culture as well as their potential to differentiate into all adult cell types. However, significant cell manufacturing challenges must be overcome before clinical application of ESC therapies will be viable. Typically in lab scale culture, health of ESC colonies is assessed by morphological evaluation which varies from person to person depending on training and experience. This large variance in culture assessment may in part contribute to the large variation in protocols and results seen throughout publications. Other than morphological evaluation, very little is done to assess cell health during culture periods. Only after the experiment do groups assess cell pluripotency (or lack thereof) via destructive measures of fixing and staining rendering the cells useless for further applications. Here we investigate an alternative approach of non-invasive image analysis utilizing fractal geometry, whereby cell growth and differentiation are indicated by cell culture morphology and represented as a numerical value. Additionally, a relationship between mass transfer within static cultures and the resulting fractal geometric values is determined. Further *to* this we are also investigating whether similar techniques can be applied to suspension culture conditions to monitor and predict culture output.

42. Immunoaffinity Aqueous Two-Phase System Bioengineering Strategies for the Potential Recovery and Purification of Stem Cells

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During the past decades, stem cells transplantations have awakened great interest in clinical research thanks to the potential results that can be achieved when employed as a therapeutic alternative for several incurable chronic and degenerative diseases. These expectations arise from the unique characteristics these cells possess, such as self-renewal and their multipotent long term differentiation potential.

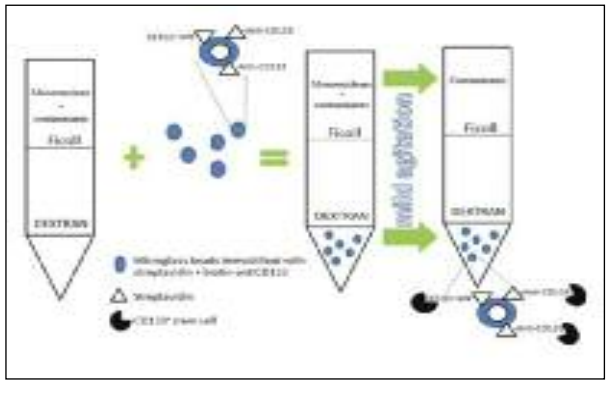

Figure 1. Schematic diagram of the proposed strategy exploiting the addition of immobilized microglass beads to the ATPS.

In this sense, the recovery and purification of stem cells is crucial in order to supply the number of cells required and in the degree of purity needed. In other words, the success of a transplant procedure is to a large extent dependent on the effectiveness of the purification and recovery steps. A desirable recovery method for

stem cells has to guarantee high purity and should be sensitive, rapid, quantitative, scalable, non-or minimally invasive in order to preserve viability and differentiation capacity of the purified cells. Currently, there are a wide range of methodologies available for stem cells isolation. Nevertheless, there is not a golden standard method that accomplishes all requirements (1).

Immunochemical affinity has become one of the most exploited techniques for stem cells purification, due to the high specificity conferred by the surface marker (cluster of differentiation CD) they employ as molecular tagging. For example, one of the most recently used CD for identification of stem cells is the novel CD133. CD133 or human prominin-1 is a five-transmembrane hematopoietic stem cell antigen (2) that appears to be a reliable marker for the isolation of neural stem cells (3) and has the ability to promote neural growth (4). Particularly, researchers from the Hospital San José Tec de Monterrey have isolated CD133+ stem cells and transplanted them into the frontal motor cortex in amyotrophic lateral sclerosis (ALS) patients (4). ALS is a neurodegenerative disease characterized by the rapid weakening and selective death of neurons. However, the current purification techniques employed for stem cells treatments are limited by their potential scale up feasibility, resulting in a non-generic process application.

In this context, aqueous-two phase systems (ATPS) represent an attractive alternative for the recovery of stem cells. ATPS is a liquid-liquid extraction technique that exhibits several advantages including: biocompatibility, economically attractive, scalable, and low processing time (5). Moreover, if this methodology is complemented with the use of antibodies (known as immunoaffinity ATPS), a novel strategy for the purification of $CD133^+$ stem cells satisfying the requirements previously mentioned, could be achieved.

The objective of this investigation is to develop a novel purification bioprocess for the selective recovery of $CD133^+$ neural stem cells employing immunoaffinity aqueous two-phase systems in order to replace the actual experimental protocol (Percoll gradient and MiniMACS separation column) for amyotrophic lateral sclerosis treatment.

The first proposed strategy involves to prove the viability of $CD133^+$ stem cells in novel ATPS composed of UCON and to characterize the partitioning behavior of the target product and contaminants in these types of systems. The second approach includes the implementation of immunoaffinity ATPS, in one of its multiple variants, in order to concentrate the contaminants and the stem cells of interest in opposite phases. For example, the addition of PEGylated CD133 to the system with the aim of obtaining the specific partitioning of $CD133^+$ cells into one of the phases. The PEGylated antibody is obtained through a site-specific PEGylation of CD133 via streptavidin-biotin conjugation (6). Another possibility is to construct ATPS composed by Ficoll 400,000 and Dextran 70,000 to which micro-glass beads immobilized with the CD133 antibody are introduced. As illustrated in Figure 1, the product of interest and the contaminants partition into the Ficoll rich top phase. After the addition of the immobilized micro-glass beads, the $CD133^+$ stem cells would ideally get attached to the beads and could be recovered in the bottom phase. After the objective is accomplished, the next step, if required, is to detach the cell of interest from the separating agent via trypsinization, in order to obtain a product suitable for a patientxs treatment.

Even though the method reported by Martinez and co-workers (2009) obtained successful clinical results, a latent niche exists for the development of a faster, scalable and cost-effective procedure that guarantees the purity and yield required for the final application of the process. In this sense, immunoaffinity ATPS represent an alternative technique that can meet these necessities making it more advantageous and viable for clinical use, thus promoting the acceleration of the widespread application of stem cells therapy. In this sense, a novel stem cell separation bioprocess based on immunoaffinity aqueous two phase system is proposed to isolate and purify $CD133^+$ stem cells from umbilical cord blood mononuclear cells.

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43. Inducible Fluorescence Exchange with Conditional Gene Over-Expression In Human Stem and Primary Cells by Zinc Finger Nuclease Mediated Genetic Engineering

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The study of the genetic basis of human development and disease has been integrally tied to *in vitro* cell culture models of embryonic and induced pluripotent stem cells. Coupled with the emergence of these cell types, methods in genetic engineering and gene manipulation have contributed to the elucidation of mechanisms underlying stem cell pluripotency/multipotency and differentiation in health and disease.

Methods and Results. A single polycistronic vector was synthesized to express both arms of the AAVS1 zinc finger nuclease driven by independent PGK promoters. Subsequently, a floxed dual fluorescence reporter containing a CAG driven floxed tdTomato followed by GFP was cloned into a targeting vector to enable zinc finger-mediated genomic engineering into the human AAVS1 locus. This targeting plasmid, pZDonor mTmG, was then engineered to contain a 2a element in frame with a unique XhoI motif for cloning of any gene of interest to be conditionally over-expressed stoichiometrically with GFP.

As proof of principle, a WT and constitutively active form of -catenin, which lacks Exon 3, were cloned into the mTmG2a vector and engineered into the AAVS1 locus of human embryonic stem cells. Conditional over-expression was initiated in the pluripotent state by transduction with a lentivirus expressing EF1 -cre. Quantitative PCR showed cell-autonomous up-regulation of -catenin target genes only in GFP+ cells from the -catenin^{DExon3} cell line.

Conclusion. This system provides a versatile method for site-specific genetic engineering of human cell lines at the AAVS1 safe haven locus that couples a floxed dual fluorescence reporter with conditional gene over-expression in a cell-autonomous manner.

44. Integrated Bioprocesses for Scalable Production, Purification and Cryopreservation of iPSC-Derived Cardiomyocytes

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The production of cardiomyocytes from induced pluripotent stem cells (iPSC) holds great promise for patient-specific cardiotoxicity drug testing, disease modeling and cardiac regeneration. The successful translation of iPSCs to these fields requires the development of robust bioprocesses for the production of cardiomyocytes in high purity, consistent quality and relevant quantities. However, existing protocols for the differentiation of iPSC to the cardiac lineage are still highly variable and inefficient, hampering their implementation in the clinic and industry.

The main aim of this study was to develop a robust and scalable platform for the efficient production and purification of iPSC-derived cardiomyocytes. Our strategy consisted in designing an integrated bioprocess by combining cardiac differentiation and cell lineage purification steps in environmentally controlled stirred tank bioreactors, where the necessary conditions to control stem cell fate are perfectly tuned. In addition, novel cryopreservation strategies were developed aiming at ensuring an efficient storage of iPSC-derived cardiomyocytes after "large-scale" production. A transgenic murine iPSC line, transfected with the vector in which the promoter of the cardiomyocyte lineage marker, α-myosin heavy chain, drive both GFP and puromycin resistance gene expression, was used to establish the utility of this bioprocess. Different bioprocessing parameters were evaluated and the results showed that pO_2, pH and stirring profile are important parameters affecting cardiac differentiation of iPSCs. The incorporation of a perfusion system in the bioreactor is being evaluated, providing a promising tool to facilitate and improve the purification of iPSC-derived cardiomyocytes. Moreover, an efficient method for the cryopreservation of a monolayer of pure cardiomyocytes was successfully developed. The use of CryoStorTM solution represented a considerable improvement of the cryopreservation process, allowing higher cell viabilities and recoveries (>90%) after thawing when compared to the standard medium formulations (<20%).

The integrated bioprocess developed in this work provides important insights for the establishment of more robust iPSC production platforms, hopefully potentiating the implementation of novel cell-based therapies.

45. Inverse Agonism of Sphingosine 1-Phosphate Receptor Three Mobilized Hematopoietic Stem Cells with Long Term Engraftment Capability

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The use of peripheral blood as an alternative to bone marrow for obtaining hematopoietic stem cells (HSC) has been successfully established. This noninvasive procedure presents several advantages such as elimination of pain and anesthesia during cell harvest. In order to enrich peripheral blood with stem cells they need to be pharmacologically mobilized with stem cell mobilizing agents. Granulocyte colony stimulating factor (G-CSF) is the gold standard of mobilizing agents but it is costly, time-consuming and does not always result in sufficient mobilization and engraftment. New strategies to mobilize sufficient numbers of HSC without impairing their functional capability would drastically improve the therapeutic use of peripheral blood stem cells. AMD3100, also known as plerixafor, is a CXCR4 antagonist that is being evaluated in conjunction with GCSF to significantly enhance stem cell mobilization. AMD3100 has not been shown to adequately mobilize sufficient numbers of HSC alone. In this work we show that inverse agonism of sphingosine 1-phosphate receptor three (S1P3) significantly mobilizes HSC from the bone marrow into peripheral blood without affecting their ability to engraft and repopulate blood cells.

Lineage 1-, Sca1+, c-kit+ (LSK) HSC were treated with VPC01211, an S1P1 agonist and S1P3 antagonist, and their chemotaxis towards SDF-1 and engraftment on marrow-derived stromal cells was assessed. Treatment abolished migration towards SDF-1 in transwell migration assays and resulted in a 33% decrease in engraftment onto marrow-derived stromal cells and a 47% decrease in engraftment when combined with AMD3100. AMD3100 was used with or without VPC01091 to mobilize stem cells in GFP+ C57Bl/6 mice. Equal volumes of peripheral blood were harvested after mobilization and used to reconstitute host C57Bl/6 mice. Chimerism in the blood and bone marrow was assessed 1, 3, 7, 14, 28 and 56 days after reconstitution. By 56 days, animals receiving grafts mobilized with both AMD3100 and VPC01091 had a 162% increase in donor content in their blood and a 63% increase in donor content in their bone marrow compared to AMD3100 alone. The fraction of donor-derived Sca1, CD45 and CD11b positive cells were also significantly increased in mice that received both compounds.

This work shows for the first time that S1P3 receptor signaling can be regulated to mobilize stem and progenitor cells from their bone marrow niches into peripheral blood to be used for stem cell therapies. The combination of AMD3100 and VPC01091 significantly enhanced hematopoietic stem cell mobilization but did not impair the functional capability of these cells. By avoiding mobilization mechanisms that are proteolytic to CXCR4, the engraftment and repopulating efficiency of mobilized cells is preserved. S1P3 receptor antagonism can be

used alone or in conjunction with compounds like AMD3100 to significantly mobilize stem and progenitor cells for endogenous and exogenous stem cell therapies.

46. Isolation and Characterization of iPSC Using a Novel Alkaline Phosphatase Live Stain

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Alkaline Phosphatase (AP) is expressed at high levels in pluripotent stem cells and has been utilized as a key early marker during somatic reprogamming. Most methods of AP staining rely on substrates that are rather toxic to cells and the stained cells can therefore, cannot be further propagated. We recently reported the development of a novel AP dye that can be utilized for live staining of pluripotent cells without altering its integrity or pluripotency.

We have further utilized the Live AP Stain to monitor the process of reprogramming of BJ fibroblasts using CytoTuneTM-iPSC Sendai Reprogramming Kit. AP Live Stain selected clones generated under feeder-dependent conditions showed embryonic stem cell-like morphology and expressed the pluripotency marker TRA-1-60. These clones expanded further and the established iPSC lines retained normal karyotype and pluripotency with marker expression and ability to differentiate into cell types representative of the three germ layers.

Resulting clones were further characterized for transcriptome using medium density TaqMan® OpenArray® qPCR platform and Illumina whole genome array. Results indicate that all the iPSC lines were similar to a control iPSC line generated using non integrational episomal vectors and H9 ESC, but distinct form parental BJ fibroblasts. However, differences were noted between different iPSC lines which were further analyzed and will be discussed.

47. Isolation and Mechanically-Induced Differentiation of Adipose-Derived Stem Cells to Improve Muscle Regeneration

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When injected as undifferentiated cells into muscle, stem cells integrate the cues around themselves to differentiate, but often for fibrotic muscle diseases that stiffen the microenvironment, e.g. muscular dystrophy and myocardial infarction, differentiation is misdirected towards osteogenesis. When co-delivered with exogenous chemical cues, bone marrow-derived stem cells (BMSC) commit to their appropriate mesodermal-derived lineages, but differentiation efficiency is not sufficiently high enough to restore muscle function. These biochemical methods overlook the biophysical interaction between cells and extracellular matrix (ECM), which is stiffened by fibrosis and clearly impacts cell function *in vivo*. Though they express a similar molecular signature and can commit to mesodermal lineages via growth factors, differences between adipose-derived stem cell (ASC) and BMSCs may likely impact their differentiation efficiency in diseased muscle. Here we found that ASCs reflect the same qualitative stiffness sensitivity as BMSCs with morphological changes reflecting differentiated phenotypes (Engler et al, Cell 2006), but quantitative analysis of lineage expression showed ASCs correctly express the appropriate temporal sequence of muscle transcriptional regulators, e.g. MyoD, Myogenin, MEF2C, etc., and do so at levels at least 10-fold higher than BMSCs. Moreover by 7 days in culture, 2.1% of ASCs form multi-nucleated myotubes with a continuous network that is not the result of misdirected cell division. This process mirrors that in primary muscle cells, and most importantly, BMSCs were never observed to undergo this process. Electrophysiological stimulation of ASC-derived myotubes is similar primary muscle cells whereas BMSC stimulation was markedly lower. Treatment with myoseverin, a microtubule depolymerizing drug that severs myotubes, could disrupt ASC-derived myotubes, but 7 days after drug washout, ASCs refused and formed myotubes at a rate similar to their pretreated value. Most encouragingly, ASC-derived myotubes, when replated onto non-permissive substrates, maintain their differentiated state, which implies that these cells injected into fibrotic muscle diseases may likely be more successful in engrafting and restoring muscle fiber function.

48. Mesenchymal Stem Cell Gene, Protein and Epigenetic Response to Shear Stress Stimulus

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Introduction: Bone marrow-derived mesenchymal stem cells (MSCs) have multilineage differentiation potential and are being considered as a possible vascular cell source for clinical cell therapies. There is evidence to suggest that MSCs can express phenotypic characteristics of vascular cells including endothelial, smooth muscle and cardiac myocyte cells. Additionally, a number of studies have focused on the potential of MSCs to treat myocardial infarction (MI) either through cell integration, differentiation to replace damaged cells or beneficial paracrine actions. Currently, the effects of biochemical and physical cues on MSC gene expression, epigenetic modifications and differentiation are yet to be well characterized. This study investigates the response of MSCs to growth factor stimuli and physiological levels of shear stress and their ability to acquire endothelial cell (EC) characteristics. As epigenetic regulation and stability play an important role in stem cell fate determination and differentiation the effects of shear stress conditions on MSC epigenetic modifications will be assessed. Further, it will be important to characterize the epigenetic changes caused by *in vitro* culture conditions to define the optimal conditions to elicit stable phenotypic alterations.

Methods & Results: Human MSCs (n=3 donors) were cultured in EC differentiation media (EC group) or maintenance media (control group) for 14 days and then seeded onto collagen IV. After 48 h both MSC groups were subjected to laminar shear stress (15 dynes/cm2 for 48 h) using a parallel plate flow chamber while static culture conditions served as controls. MSCs

were then assessed for altered gene expression in both media groups after shear or static culture (day 18) using hMSC RT-PCR arrays and quantitative RT-PCR for endothelial-like differentiation markers (PECAM-1, VE-CAD and vWF). To allow comparisons of baseline epigenetic profiles, firstly, un-treated MSC and EC samples were assessed for the levels of DNA methylation at the promoters of marker genes using bisulfate sequencing. Then MSC samples in both media conditions were assessed after shear or static culture conditions. After 48 h of shear stress, the EC group demonstrated an intact confluent cell monolayer when compared to the control group where a fraction of cell-free regions in the monolayer could be seen within the majority of samples. MSC gene profiling comparing shear vs. static culture conditions (day 18) indicated a total of ten of 84 genes tested were shear-responsive (≥ 2-fold difference, $p \leq 0.05$) in the EC media group, and seven of 84 genes in the control media group. Shear stress significantly increased vWF gene expression in MSCs pre-conditioned with EC differentiation media. Independent of media conditions, hepatocyte growth factor/scatter factor (HGF/SF), a multipotent cytokine reported to attract other stem cells, was significantly decreased under shear conditions and exhibited the greatest gene fold regulation (>15 fold). MSC HGF production at the protein level was further investigated in the spent media using enzyme-linked immunosorbent assays (ELISAs). HGF content within the spent media samples was significantly decreased under shear stress conditions for all donors when compared with static controls. DNA methylation analysis, by bisulfite sequencing, of the vWF promoter demonstrated that the promoter is unmethylated in ECs, but fully methylated in MSCs. Whilst vWF is activated under shear stress, vWF promoter remains largely methylated under the treatment. We are currently investigating whether shear stress combined with DNA methylation blocking agent, such as AzaC, will increase the efficiency of differentiation of MSCs toward EC cells.

Conclusions: This study has demonstrated that biochemical and physiological stimuliinduced altered MSC gene expression levels; however, there was limited response of the EC-associated genes being up-regulated and no alterations to vWF DNA methylation patterns were detected. Shear stress also affected MSC secretion of HGF, suggesting shear stress conditions may affect other cytokines produced by MSCs and hence may influence their paracrine actions. When considering a bioreactor environment, cells will likely be exposed to shear stress conditions hence further investigations to determine the influence of shear stress on MSC cytokine production, and epigenetic features, including DNA methylation modifications of marker genes, is required. This will aid in engineering the optimal microenvironment in a bioreactor and in yielding clinically effective MSCs.

49. Microfluidic Control of Three Dimensional Embryonic Stem Cell Microenvironments

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Cell specification during embryonic development is orchestrated by precisely controlled spatial and temporal signaling arising from the development of morphogen gradients via paracrine signaling (Murry et al. 2008). Although the morphogenesis which occurs within embryoid bodies (EBs) is often heterogeneous and lacks the spatial precision exhibited *in vivo*, embryonic stem cells (ESCs) retain the capacity to respond to morphogenic cues. Although the phenotype of ESCs may be altered by morphogen delivery to cell monolayers, the culture of ESCs as three-dimensional aggregates can more accurately recapitulate cellular adhesions and signaling exhibited by stem cells found in native tissues (Akins et al. 2009). Specifically, the differentiation of ESCs as multicellular aggregates may enable the three-dimensional assembly of intercellular adhesions, including cadherins and connexins, which are important for morphogenesis. However, efficient directed differentiation of ESCs as aggregates is technically challenging, as morphogen delivery is often constrained to the exterior cells due to diffusive transport limitations (Sachlos et al. 2008; Carpenedo et al. 2009). The objective of this work was to develop a microfluidic approach to spatio-temporally control morphogen delivery within the three-dimensional ESC microenvironment, in order to enable efficient directed differentiation of ESCs.

The size of EBs and formation kinetics were precisely controlled by the forced aggregation of ESCs via centrifugation into 400 μm diameter micro-wells. After 24 hours of formation, uniform EB populations were transferred to rotary orbital suspension culture at 45 rpm, whereby morphogenesis was controlled by inhibiting subsequent agglomeration of individual EBs. The mechanical properties of EBs were analyzed using a Microsquisher (CellScale), which is a micron-scale material tester that exerts compression forces via a cantilever, while measuring force as a function of displacement. Single EBs were compressed under constant strain, force (creep), or displacement (stress relaxation). Additionally, a custom bioreactor was developed, which enables controlled convection by altering the pressure drop across EBs. Using this bioreactor setup in parallel with a pressure transducer, the pressure drop was measured at a range of volumetric flow rates (100-1000 μL/min), in order to determine the fluidic resistance and permeability of EBs. Additionally, EBs were maintained under continuous perfusion (1 μL/hour), and the expression of Brachyury-T was monitored via flow cytometry.

In contrast to static cultures, whereby individual EBs agglomerated to form large, irregularly shaped aggregates, culture of EBs on rotary orbital shakers enabled the maintenance of homogeneous populations which exhibit similar sizes, as well as total cell yields per EB, across a range of rotary conditions (45-65 rpm). Additionally, EBs seeded with different cell numbers (500-2000 cells per EB) maintained statistical differences in relative size over the culture period in rotary suspension. Together, the combination of microwell formation and rotary orbital suspension culture yielded uniform populations of EBs, ultimately enabling more systematic analysis of the dynamics of individual EB microenvironments during the course of ESC differentiation. In order to characterize the impact of EB morphogenesis and remodeling on the physical properties of the EB microenvironment, the mechanical and transport properties were measured as a function of differentiation stage. Overall, the elastic modulus of EBs significantly increased ($p=0.029$) after 7 days of differentiation (9.81 +/- 1.76 Pa) compared to day 2 (4.08 +/- 0.96 Pa), and EBs exhibited changes in viscoelastic properties, including decreased creep and increased stress relaxation responses. Additionally, the pres-

sure drop across EBs was measured to calculate the fluidic resistance and fluorescence recovery after photobleaching (FRAP) was used to measure the attenuation of diffusion inside of EBs. Together, the fluidic resistance increased, concomitant with decreased permeability (4.93 ± 0.49 x10-12 m2 after day 3 compared to 1.35 0.31 x 10-12 m2 after 9 days of differentiation; p=0.004), and diffusivity (0.97 ± 0.02 after 2 days compared to 0.84 ± 0.01 after 7 days; p<0.001) during the course of EB differentiation, which indicates that coincident changes in EB structure appear to increasingly restrict transport, especially as differentiation proceeds.

The results from these studies collectively indicate that the transport and mechanical properties of EBs are altered as a result of morphogenic and phenotypic changes that arise during ESC differentiation. Our data thus far suggests that EB transport limitations can be attenuated by convective transport via microfluidic perfusion, which may enhance the efficiency of directing differentiation towards specific cell phenotypes and germ lineages. Ultimately, this work addresses the requirements for engineering 3D tissues from stem cells, in order to create scalable culture platforms of multicellular assemblies and tissue constructs for regenerative medicine therapies.

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50. A Microfluidic Platform for hESC Differentiation Into Hepatocytes

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Hepatotoxicity is a major factor contributing to the high attrition rate of drugs. At least 25% of the drugs are prematurely terminated or withdrawn from the market due to liver toxicity. Primary human hepatocyte culture is currently used for studying drug metabolism and liver toxicity *in vitro*. However, the use of human hepatocytes as a cell-based model during the early stages of drug discovery is limited by donor organ availability, variability with respect to cell attachment and function, as well as the difficulties inherent in maintaining hepatocytes in primary culture. The promise of stem cell derived hepatocytes as a renewable cell source, with consistent cell properties, holds great hope and expectation in pharmaceutical development. Even though researchers have been striving for differentiation from human embryonic stem cells (hESCs) into hepatocytes vigorously, conditions for directing hESCs to hepatocyte lineage with homogeneity and high hepatic function are not yet fully defined. Directed differentiation of human embryonic stem cells (hESC) into hepatocytes using 2D culture models has resulted in low and incomplete hepatocyte function. The incomplete differentiation may be due to a limitation of the 2D culture model. For example, cells due not assume a normal cell shape, are not exposed to physiological stimuli such as shear and be differentiation may be adversely affected by uncontrolled fluctuations in to nutrients, growth factors, etc. In this study we used a microfluidic approach to generate hESC derived hepatocytes to better model the dynamic physiological environment of cells in the body. The cells in the perfusion device formed 3D organoids structures, experienced shear stress and were provided a constant supply of nutrients, etc while removing waste products. Using a microfluidic culture device allowed better chemical, mechanical and temporal control of the differentiation process and resulted in enhanced differentiation and maturation of stem cell derived hepatocytes.

Previously Corning scientists successfully cultured human primary hepatocyte in a microfluidic device which promoted and maintained 3D tissue-like cellular structure and cell-specific functionalities (e.g. restoration of membrane polarity and hepatocyte transport function). From this perspective, we designed a similar device for hESC differentiation. The master mold for the device was fabricated by photolithography methods. The device was then produced by molding PDMS against the master mold. The device had multiple perfusion channels where there were two parallel flow channels on either side of the cell chamber. The cell retention chamber is 300 um wide and 100 um high. Cells were loaded to the device through tubing. Medium supplements with different growth factors are added to the device constantly via perfusion channels at the flow rate of 12 ul/hr.

The TW6 human embryonic stem cell line, derived by Industrial Technology and Research Institute (ITRI), was used in this study. Hepatic maturation was monitored by staining for alpha-fetoprotein (AFP) and albumin (ALB), fetal and adult liver markers, respectively. TW6 cells were treated with activin A for three days followed by FGF4 and HGF for another 5 days in 2D culture. Cells were released and seeded into the microfluidic device and continuously perfused with culture medium for ten days (e.g. day 8 to day 18). Control cells were maintained in 2D culture. At day 13, most cells in 2D culture were defined as hepatocyte progenitors (AFP+/ALB-) while cells cultured in the device also began to express albumin. Comparative phenotypic characterization of liver cells at day 18 revealed that cells maintained in the microfluidic device matured more rapidly as evidence by their complete lost of AFP expression while high level of albumin expression. Cells in 2D culture were AFP+ and ALB+.

Our findings suggest that directed differentiation of hESC in a microfluidic device may provide a promising approach to effectively derive mature hepatic cells. In contrast to conventional monolayer culture, the device provides a controlled, dynamic 3D environment that enhances the differentiation kinetics as well as the maturation of stem cell-derived hepatocytes.

51. Microfluidic Single-Cell Analysis of Embryoid Body Heterogeneity

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Pluripotent stem cells exist as a heterogeneous cell population with unique molecular properties and functional characteristics. Embryonic stem cells are often cultured as embryoid bodies (EBs), a platform which allows for spontaneous differentiation and results in phenotypic heterogeneity in the cell population. Significant research has been conducted to examine the ability of different biochemical and environmental factors to direct EB differentiation. For example, our lab has shown that changes in the hydrodynamic environment of EBs can influence differentiation efficiency, yield, and homogeneity (Carpenedo et al. 2007). One of the problems encountered in the work is the lack of capable analysis methods to determine characteristics of the individual cells that make up a single EB. Existing methods, such as flow cytometry, provide a high-throughput means to analyze phenotypic characteristics of a cell population but require cell quantities that are much greater than the number of cells comprising a single multicellular aggregate. Flow cytometry also does not provide spatial information regarding intracellular molecular localization. Conversely, confocal imaging provides spatial information, but it is low-throughput and has limited capability to image more than 50 µm into an embryoid body. Tools that increase understanding of when and where heterogeneity is occurring in a cell population can lead to better assessment of directed differentiation techniques. Therefore, the objective of this study was to develop a microfluidic approach to analyze the individual phenotypes of the cells from single EBs.

A microfluidic device was designed to allow for high-throughput capture and imaging of up to 4,000 single cells in parallel (Chung et al. 2011). Polydimethylsiloxane (PDMS) devices were fabricated via soft lithography rapid prototyping and replica molding, followed by plasma-bonding onto glass slides. Cells were loaded into the device by pipetting a cell suspension into an inlet punched with a 19-gauge needle. The cells are transported through the device by gravity-driven flow, resulting in low flow rates and shear stresses.

Embryoid bodies were formed by forced aggregation of murine ESCs (D3 line) via centrifugation into 400 µm diameter PDMS micro-wells. To control the size of the EBs, different initial seeding densities (150, 500, and 1500 cells per well) were employed. After 20 hours, the resultant population of EBs was transferred to suspension culture (approximately 2000 EBs per 100 mm dish) and placed on rotary orbital shakers at a speed of 40 rpm until time points for analysis were reached. Prior to cell loading in microfluidic device, EBs were dissociated using 0.25% trypsin-EDTA and incubated in 3 mM EGTA to inhibit intercellular adhesions. A live/dead cell assay confirmed that this procedure did not compromise cell viability. The cells were loaded into the device as described above, followed by perfusion of a 4% paraformaldehyde solution through the device to fix the cells. Cell-laden devices were stored at 4°C until immunostaining was performed. To examine cellular phenotype, the microfluidic devices were perfused with primary antibody solutions for the pluripotency markers Oct4 and SSEA1. Following perfusion of corresponding secondary antibody solutions, the devices were imaged using a fluorescence microscope. Image processing using Image J was performed to determine the fluorescent intensity for each individual cell. The fluorescent intensity was normalized back to the values for the parent undifferentiated cell population.

Initial studies were performed with a population of EBs dissociated prior to loading into the device. Loss of the pluripotency markers Oct4 and SSEA1 was examined for EBs of different sizes. As expected, a decrease in fluorescent intensity was observed with increased time in embryoid body culture. After nine days, EBs formed with 150 cells exhibited a 92% decrease in Oct4 expression, compared to 67% and 74% decreases for EBs formed with 500 cells and 1500 cells, respectively. In addition, EBs formed with 150 cells exhibited a 0.2% decrease in SSEA1 expression, contrasted with 34% and 48% decreases for EBs formed with 500 cells and 1500 cells, respectively. To examine the diversity in marker expression, the frequency distribution of fluorescence intensities was determined for individual cells. The heterogeneity of marker expression was quantified using the Shannon Diversity index. A minor correlation between the size of the EB and the heterogeneity of SSEA1 expression was observed after nine days, with smaller EBs exhibiting a more heterogeneous expression pattern than larger EBs. These initial results indicate that the microfluidic device can provide information regarding both the level and heterogeneity of expression in single cells. In addition, the use of this tool has uncovered a potential relationship between EB size and the heterogeneity of pluripotency loss.

Preliminary analysis of single embryoid bodies has been performed in the device, an approach which can improve knowledge regarding the heterogeneity of the cell population within individual EBs. The cell capture efficiency for a single EB was determined by dissociating a single EB, estimating the starting concentration of cells using a hemocytometer, and counting the number of cells successfully loaded into the microfluidic device. The single EB cell capture efficiency after all transfer steps was found to be 45%, indicating that despite the difficulty of working with such low cell populations, the device is an effective tool for single EB analysis. Ongoing studies will compare the effectiveness of the microfluidic device to determine the coordinated loss of the pluripotent transcription factors Oct4, Nanog, and Sox2 in cells from individual EBs to conventional methods, such as flow cytometry and confocal microscopy.

Taken together, these results suggest that the microfluidic cell trap device is capable of elucidating new information regarding the heterogeneity of protein expression in individual embryoid bodies. This work highlights the need for single cell analysis techniques in order to assess heterogeneous cell types, particularly pluripotent stem cells. The use of this device can provide better evaluation about the efficacy and efficiency of directed differentiation methods by parsing out single cell dynamics from broad population-based information.

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52. Micropatterend Gel Controlled Tubulogenesis From Dispersed Ureteric Epithelial Cells

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Introduction: Renal development is initiated by interaction of the ureteric bud with the metanephric mesenchyme. We aimed to generate a tubular structure from dispersed renal progenitor cells *in vitro* using a micropatterned gel to control growth and geometry of the structure.

Methods: A micropatterned agarose gel was cast from a silicone mask, in which the positive shape of the pattern was etched by photolithography. The pattern in the agarose gel produced, contained rectangular cavities (3mmx150µmx150µm), into which dispersed mouse ureteric bud cells (CMUB-1 or mIMCD) suspended in collagen I were seeded by centrifugation. The gel holding the cells was subsequently cultured in DMEM+10% FCS.

Results: After 24h, the embedded dispersed ureteric bud cells formed single layered tubular structures that contained a lumen, as viewed under confocal microscope with laminin staining. The structures conformed to the shape and size of the cavities in the gel, and detached from the mold after formation. The terminal ends of the tubular structure were multilayered and closed. Growth factors EGF, FGF, HGF or the addition of aldosterone, did not influence tubular formation. Studies are underway to examine the effects of GDNF on directional growth and branching of the generated tubules.

Conclusions: We conclude that micropatterned gels can be used to control the growth of geometrically defined structures of the developing kidney from dispersed cells. These structures can be used as elements for kidney neo-organogenesis and renal development studies *in vitro*.

53. Modeling a Human Genetic Disorder of the Autonomic Nervous System In Induced Pluripotent Stem Cells

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Congenital Central Hypoventilation Syndrome (CCHS) is a rare disorder of respiratory control that shows significant neural crest (NC)-pathology, affecting cranial, cardiac, and trunk NC cell lineages. CCHS is highly correlated with mutations in the PHOX2B, a paired-like homeodomain transcription factor. CCHS-associated PHOX2B mutations show preservation of the DNA homeodomain structure, and therefore may continue to bind DNA; however, how this interaction affects gene regulation and ultimately cellular function remains unknown. Interestingly, PHOX2B mutations also cause a NC tumor (Neuroblastoma), and it is unclear how this tumor would be related to CCHS. We have successfully generated induced pluripotent stem (iPS) cells derived from a CCHS autopsy subject and can differentiate these cells into NC cells. CCHS-derived NC cells show a hyper-proliferative and abnormal migratory phenotype. Currently, we do not know which NC cell subpopulation contributes to the hyper-proliferative and/or migratory phenotype, since unique gene signatures of NC subpopulations remain unknown. To identify the unique gene signatures of NC subpopulations we are performing RNA-seq from genetically marked NC cells in mouse embryos, as well as in human iPS-derived NC cells using several different NC differentiation methods. With unique gene signatures, we can then determine the contribution of each NC cell subpopulation in CCHS and hopefully improve regenerative therapies and tissue repair with implications for multiple NC diseases.

54. Modeling Congenital Central Hypoventilation Syndrome (CCHS) Using Induced Pluripotent Stem (iPS) Cells

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Congenital Central Hypoventilation Syndrome (CCHS) is a rare disorder of respiratory control that shows significant neural crest (NC)-pathology. CCHS is highly correlated with mutations in the PHOX2B gene. PHOX2B is a paired-like homeodomain transcription factor. CCHS-associated PHOX2B mutations show preservation of the DNA homeodomain structure, and therefore may continue to bind DNA; however, how this interaction affects gene regulation remains unknown. The ultimate goal of this project is to generate an *in vitro* model of CCHS using induced pluripotent stem (iPS) cells derived from a CCHS autopsy subject that suffered from a severe case of intestinal agangliaonosis. To this end, we validated full cellular reprogramming of fibroblasts by molecular marker expression. Furthermore, we differentiated the PHOX2B mutated iPS cells into all three human germ layers. With these cell lines, we hope to test the hypothesis that aberrant NC cell migration leads to CCHS.

55. Multifactorial Analysis of Embryonic Stem Cell Self-Renewal Reveals a Crucial Role of GSK-3-Mediated Signaling Under Hypoxia

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Work previously performed in our group showed that culturing mouse embryonic stem (mES) cells under different oxygen tensions gave rise to different cell proliferation patterns and commitment stages depending on which signaling pathways are activated or inhibited to support mES cell self-renewal. These findings indicate that mES cell self-renewal and pluripotency, which are dependent on multifactorial signaling networks, can be influenced by different oxygen levels. However, a clear understanding of the molecular mechanisms that regulate stem cell fate and

function under these conditions is not well understood.

To elucidate and dissect how each signaling pathway is functioning at physiological and non-physiological oxygen tensions, we have used a multifactorial approach and response surface methodology. The sole and interactive influence of MEK/ERK pathway inhibition, activation of Wnt/β-Catenin by GSK-3 inhibition, and activation of LIF/STAT3 signaling, was statistically evaluated during expansion of mES cells at different oxygen tensions using a factorial design. Collectively, this approach provided new insights into the mechanisms by which oxygen influences mES cell self-renewal and pluripotency while distinct pathways are activated or inhibited. This modeling approach revealed that at lower O2 tensions LIF/STAT3 signaling and Wnt/β-Catenin, in particular, show a significant role towards maintenance of mES cell self-renewal and pluripotency. Our results add new insights into the mechanisms by which oxygen tension influences mES cell fate, and GSK-3 inhibition in particular showed an important role towards maintenance of ES cell pluripotency.

56. Myosin-II Is a Central Node for Physical Regulation of Adult Hematopoiesis

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Activation of hematopoietic stem cells (HSCs) and progenitors in a bone marrow niche involves cytokinesis and motility processes that in other cell types requires cytoskeletal contractility. Here, enrichment of long-term human HSCs by up to 20-fold from a mixed culture of CD34+ human bone marrow cells is achieved through reversible inhibition of Non-muscle myosin-II (NMM-II) which frustrates cytokinesis of rapidly proliferating progenitors. HSC pathways are largely preserved in culture as elaborated by high-accuracy titration microarray analyses of ~1000 recognized hematopoietic genes that also reveal transient expression of NMM-IIB, an isoform previously unknown with HSCs. NMM-IIB has been implicated in firm attachment, consistent with anchorage in a niche, whereas constitutive NMM-IIA is regulated not at the level of expression but by tail phosphorylation that deactivates this isoform. Functional analyses elucidate key roles in cortical rigidity and mechanosensing, which is evident with HSC numbers increasing on soft matrix at high ligand. Long-term human HSCs from myosin-inhibited cultures prove as functional in the marrows of xenografted mice as freshly derived HSCs, but expression profiles are sufficiently distinct – especially when compared with progenitors which fail to engraft – that a small subset of genes emerge as critically 'up' for engraftment, including NMM-IIB. Furthermore, while myosin inhibition generally suppresses progenitors, megakaryocytes increase in multinucleation, with xenografts showing 4-fold more circulating platelets *in vivo* within the first week. Myosin-II is thus a multifunctional node in contributing to division, adhesion, and rigidity in HSC differentiation.

57. Neural Crest-Derived Dental Pulp Stem Cells Function As Ectomesenchyme to Support Salivary Gland Tissue Formation

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Xerostomia, dry mouth due to loss of functional salivary gland, is caused by Sjögren's syndrome, radiotherapy for head and neck cancer, medications and aging, leading to patients' suffer from difficulties in swallowing and speech, as well as oral diseases. Stem cell therapy is considered a potential therapeutic alternative. However, combinatory approaches including not only salivary gland stem cells but also supportive cells and appropriate extracellular matrix are necessary to form a functional salivary gland. Like tooth formation, the development of salivary gland requires epithelium interacting with neural crest-derived mesenchyme. Dental pulp stem cells (DPSC) isolated from murine dental pulp are neural crest-derived. Herein, we used the human salivary gland (HSG) cell line as a model to study the effects of DPSC on salivary gland differentiation. Upon *in vitro* differentiation on Matrigel, HSG alone and HSG co-cultured with Wnt1-Cre/R26R-LacZ derived DPSC (HSG+DPSC) differentiated into acinar-like structures. However, HSG formed more mature (higher expression of LAMP-1 and CD44), larger and increased numbers of acinar structures in HSG+DPSC. *in vivo* subcutaneous co-transplantation of HSG and DPSC with hyaluronic acid (HA) hydrogel after 2 weeks was evaluated by Q-RT-PCR, morphological and immunohistological assessment. Compared to HSG transplants which only showed undifferentiated tumor-like cells, HSG+DPSC demonstrated (1) higher expression of murine mesenchymal marker Fgf-7 (2) higher expression of mature human salivary gland differentiation marker alpha-amylase-1 AMY-1 (3) higher expression of murine endothelial, vWF, neuronal, NF-200, and angiogenic markers, Vegfr-3 and Vegf-C; (4) mucin-secreting acinar- and duct-like structures with abundant blood vessels at the interface with DPSC; and (5) more mature glandular structures double-positive for salivary gland differentiation markers CD44 and LAMP-1. These results indicate that DPSC supported and enhanced HSG differentiation into functional salivary gland tissue. This study illustrates the potential of DPSC as inductive mesenchyme for salivary gland regeneration, repair, and tissue engineering.

58. A Novel Method for Fabrication of Chemically Heterogeneous 3D Nanofibers Scaffold for Tissue Engineering Applications

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The aim of the tissue engineer to develop functional scaffold with adequate close mimic of highly heterogeneous physical (topography) and chemical (functional groups and molecules like proteins etc.) features of natural extracellular matrices (ECM) to encourage cells for regeneration of the specific functional tissues, is obligatory and challenging. Most of the current scaffold fabrication techniques have been focused to mimic the physical structure of natural ECM (i.e., topological features) in the scaffold. Conversely to introduce the chemical feature into the scaffold, most common post chemical modification of fabricated scaffold has been used. In this process it is difficult to introduce multi chemical functionality into a scaffold. Thus the current scaffold fabrication methods have limitation in fabrication of 3D scaffold having both physical and heterogeneous chemical features in a scaffold. We have developed a novel fabrication technique of nanofiber-pocket 3D scaffold where each fibers pocket (>100 μm) in 3D can have different physical and chemical signatures. This technique has four distinct process steps; nanofibers preparation, chemical modifica-

tion of the nanofibers, protection of the fiber by encapsulation into NaCl crystal, and arranging the fibers in 3D. The nanofibers are encapsulated into the NaCl crystal to protect from solvents and the encapsulated fiber-NaCl crystals are used for 3D scaffold fabrication by salt-leached techniques. The NaCl crystals protect nanofibers from array of organic solvents and preserving their physical and chemical signature during the scaffold processing and carry into the 3D scaffold. This is a generic technique that allows us to fabricate a 3D nanofibers scaffold of single or multiples polymers systems with chemical heterogeneity (encapsulated/immobilized growth factors, proteins and biomolecuels for specific cell functions and fate) in 3D. The scaffold prepared by this technique has superior mechanical and cell penetration properties than the conventional electrospun nanofiber scaffold. This technique allows us to prepare scaffold with specific chemical/cellular functionality in 3D. For an example, we prepared scaffold with/without fibronectin coated fiber pocket arranged in 3D for targeting the stem cell to the site of fibronectin coated fiber pocket of the scaffold in 3D.

59. Novel Strategies for 3D Neural Culture and Gene Delivery: Human Central Nervous System *in vitro* Models for Preclinical Research

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The development of new drugs for human Central Nervous System (CNS) diseases has traditionally relied on 2D *in vitro* cell models, that fail to recapitulate the characteristics of the target tissue, and genetically engineered animal models, which often diverge considerably from the human phenotype, contributing to a high attrition rate - only 8% of CNS drugs entering clinical trials end up being approved. Human 3D *in vitro* models are useful complementary tools towards more accurate evaluation of drug candidates in preclinical stages, as they present an intermediate degree of complexity between the traditional 2D monolayer culture conditions and the brain.

Herein, we describe a robust and reproducible methodology for the generation of 3D *in vitro* models of the human CNS following a systematic technological approach based on stirred culture systems and using human neural stem cells (hNSC) as a scalable supply of neural-subtype cells. We took advantage of midbrain-derived hNSC commitment to the dopaminergic lineage to generate differentiated neurospheres enriched in dopaminergic neurons. Control of chemical and physical environmental parameters allowed for the differentiation into neural-subtype cells in reproducible ratios. Detailed cell characterization of differentiated neurospheres was performed along culture time using spinning disk confocal microscopy, field emission scan electron microscopy (FESEM), transmission electron microscopy (TEM), qRT-PCR and Western Blot.

To develop the full potential of this human CNS model system it is of paramount importance to establish reliable and robust methodologies for gene transfer and manipulation of gene expression. To address this issue we have used canine adenovirus type 2 (CAV-2) viral vectors, that present high cloning capacity, long-term transgene expression and low immunogenicity and have been shown to preferentially transduce neurons. Transduction of differentiated neurospheres was optimized using a CAV-2 vector carrying eGFP reporter gene attaining efficient gene transfer and stable expression of the transgene for at least 10 days.

The model system developed in this work constitutes a practical and versatile new *in vitro* approach to study human CNS, and is expected to increase the relevance of *in vitro* preclinical research of human CNS disorders. Furthermore, this culture strategy may be extended to other sources of human neural stem cells, such as human pluripotent stem cells, including patient-derived induced pluripotent stem cells, broadening the applicability of these models even further.

60. A Novel Strategy to Genetically Engineer Neural Stem Cells

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Neural stem cells (NSC) hold a great potential for the development of gene and regenerative therapies for the treatment of neurodegenerative disorders and brain cancer. These cells can be genetically modified *in vitro* to express desired transgenes for an increased expansion or differentiation capacities, as well as to be used *in vivo* as cellular carriers of toxic payloads for tumor elimination or growth factors for cellular regeneration in the Central Nervous System (CNS). The method of choice to genetically modify these cells are based on the use of viral vectors but, due to safety concerns, non-viral methodologies are gaining a major impact mainly in clinical settings. A new generation of DNA vectors lacking the bacterial backbone, named Minicircles (mC), has been recently tested in mammalian and stem cells with superior results in terms of transfection efficiency and long-term expression of the transgene, when compared to conventional plasmids. Actually, in Gene/Cell Therapy, when the goal is the transient expression of a specific therapeutic protein, it may be beneficial to extend its period of expression.

The main purpose and novelty of this work was the evaluation of the effect of mC on embryonic stem cell-derived Neural Stem Cells envisaging a safe strategy for future gene/cell therapy applications for the treatment of neurological diseases. Two different non-viral methods, microporation and lipofection, were used to transfer mC into mouse embryonic stem cell-derived NSC, mC being more efficiently transferred into NSC by the first method. Around 80% and 25% of transfected cells was achieved with microporation and lipofection, respectively, with cell viabilities ranging from 85-95%. Interestingly, when comparing mC with the respective conventional plasmid DNA and using similar initial number of molecules of both vectors, mC promoted higher number of NSC expressing the transgene with higher cell viabilities (in both cases 10% higher), after microporation. Moreover, long term analysis showed that NSC harbor a higher number of mC copies, exhibit higher transgene expression and also that mC are less degraded when compared to conventional

pDNA. Indeed, seven days after microporation, around 35±7% and 23±1% of cells were still expressing the transgene, when using mC and pDNA respectively, most probably due to the presence a higher number of mC molecules (~2500 copies more) that were quantified inside NSC's nucleus. We also verified that microporated NSC maintained their ability to differentiate into astrocytes and neurons. Taken together, our results offer the first insights on the use of microporation and mC as a novel and safe strategy to genetically engineer NSC envisaging their use in clinical settings for the treatment of neurodegenerative diseases.

61. Qualification of 19F MRI to Detect CD34+ Hematopoietic Stem Cells *in vivo*

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Non-invasive detection and tracking of stem cells post-delivery lends insightful information into the mechanism of action of an intended therapy and/or possible reasons for therapeutic failure. Since the biological and physical makeup of the therapeutic cells is often quite similar to the host tissue, a chemical moiety is often needed to supply contrast or signal to some external physical method of detection. Thus, rigorous examination of the effects of the contrast agent on the cells in question must be performed to ensure minimal disruption to the therapy. In this work, we examine the effects of a novel, self-delivering 19F MRI contrast agent on hematopoietic stem cells (HSC) as well as the ability of MRI to detect cells *in vivo*.

Due to the nature of the contrast agent used, cellular labeling simply consists of co-incubation of the reagent and the cells of interest; the reagent concentration and incubation duration are parameters available for optimization of cellular viability, labeling uptake, and, if needed, cellular function post-label. Comparison of 19F-labeled HSC and unlabeled control cohorts in *in vitro* colony forming assays resulted in equal numbers of total colony forming units (CFU), as well as individual CFU types, indicating that label presence did not effect multipotency. Also, a cobblestone forming assay provided evidence that the concentration of stem-cell regenitors did not change upon labeling. *In vivo* reconstitution studies in mice, using labeled and unlabeled murine bone marrow HSC, resulted in equivalent development of CFU in the spleen, as well as reconstitution of the lymphoid and myeloid compartments. The fact that these highly complex processes remain largely unaltered in the presence of contrast agent provides strong evidence of the inertness of the reagent and that the therapeutic potential of the cells is likely maintained. Further, proof of principle MR imaging of injected and implanted HSC in a rat thigh was performed, indicating that HSC can be detected with MR methods with the use of this contrast agent. The data suggests that imaging data on the location and persistence of delivered CD34+ HSC can be obtained without influencing the function and/or differentiation potential of the therapeutic cells.

62. Optimisation of the Expansion and Differentiation of Embryonic Stem Cells On An Automated Microwell Platform

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Embryonic stem (ES) cell culture is currently a largely manual process, with major challenges to address in the methods used regarding scalability and variability. Process automation can be of great benefit to reduce operator-dependent variation, therefore improving cell yield and quality. This would be beneficial for production of defined cells for high throughput screening or definition of a robust cGMP process suitable for scaled-out production of cells for clinical application. This work describes the use of a fully automated Tecan platform for the hands-free expansion of mouse and human ES (mES and hES) cells, as well as directed neural differentiation of mES cells.

Key bioprocess variables were initially optimised to develop a Standard Operating Procedure (SOP) for the expansion and differentiation of mES. Comparisons between the manual and automated process were shown by expanding Oct-4-GiP mES cell line over eight passages followed by directed differentiation into neural precursors. Automated culture was shown to improve the consistency of cell yield up to 3-fold. Cells produced maintained their pluripotency and were able to form derivatives of all three germ layers. Using the platform's ability to control oxygen tension, mES cells were further differentiated into neural precursors at 2% oxygen and results compared to manual differentiation at 2% and 20% oxygen. Use of the enclosed automated platform avoided changes in oxygen tension during media changes, as occurs in manual culture. A 3-fold increase was found in cells expressing βIII-tubulin at 2% oxygen (automated) compared to 20% oxygen, as well as a 16-fold increase in cells expressing MAP2 at 2% oxygen (automated) compared to 20% oxygen.

For hES culture, standard mechanical passaging of cells can introduce variability during a process and furthermore is not suitable for the automated microwell platform. Two new cell lines were derived from mechanically passaged hES cells by adaptation to TrypLE ExpressTM, resulting in a karyotypically abnormal (Shef-3) and normal (Shef-6) cell line. Optimum growth conditions for each line were evaluated. Design of Experiments (DoE) was used to evaluate the effect of feeder cell and hES incoculation cell density (ICD). Results indicated that both TrypLE-adapted lines were capable of growing on a feeder layer ICD as low as 3,125 cells.cm-2. hES ICDs between the two lines vary greatly, with that of the Shef-6 line almost double.

An SOP for passaging of Shef-3 cells was established and optimised. The dissociation step was found to be critical in maintaining cell yield over multiple passages on the platform. This step was improved by using a non gelatin-coated tissue culture surface and increasing the dissociation time to 30 min. Cells were passaged over 5 consecutive passages. Cell yield remained stable at approximately 2 x 105 cells.well-1 and cell viability did not drop below 98%. Cells were shown to express pluripotency marker Oct-4 throughout all 5 passages. Pluripotency was further confirmed by high expression of SSEA-4 (98% and 98%) and TRA-1-60 (95 % and 82%) before and after processing respectively. Future work will involve passaging of the karyotypically normal Shef-6 line on the automated microwell platform.

63. Possibility of in vitro Adipogenic Differentiation of Human Adipose-Derived Stem Cells Under Co-Culture Conditions In the Presence of Human Mature Adipocyte

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The differentiation of human adipose-derived stem cells (hADSCs) into adipocytes was studied by co-culturing them with human mature adipocytes. The transwell was utilized in the indirect co-culture of hADSCs and human mature adipocytes, with 4 different kinds of ratios of hADSCs to mature adipocytes i.e. 1:5, 1:1, 2:1 and 5:1. After 8 days of co-culture, the Oil Red O and Trypan Blue staining were performed for the evaluation of adipogenic differentiation of hADSCs. In addition, the Oil Red O and Trypan Blue stainings, flow cytometry analysis and hoechst33342/PI double staining were carried out after 20 days of co-culture. The Oil Red O and Trypan Blue staining showed that hADSCs with high viability could not differentiate into mature adipocytes after 8 or 20 days of co-culture. However, flow cytometric analysis indicated that CD105 expression of hADSCs decreased after 20 days of co-culture. These results indicated that hADSCs after co-culture could not successfully differentiate into adipocytes. This work was supported by DUT11SM09, the NSFC30700181/31170945 and The Project-sponsored by SRF for ROCS, SEM.

64. Potential for Hydrogel Composites Containing Carbon Nanobrushes as Stem Cell Scaffolds

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The objective of this work is to examine potential uses for electrically conductive hydrogel composites as a 3D scaffold for stem cells and other clinically relevant cell lines. The composite is comprised of carbon nanobrushes embedded in a biocompatible poloxamer gel. This work assesses the ability of such composite gels to support the growth of fibroblasts and myocytes and eventually serve as a matrix to stimulate wound closure. In such a model, fibroblasts and myocytes are seeded on the hydrogel and bathed in culture medium. The experimental model assesses the ability of fibroblasts and myocytes to grow into and adhere to the gel. The work demonstrates that carbon nanobrushes can be dispersed within poloxamer gels, and that fibroblasts and myocytes can proliferate within homogenously dispersed carbon nanobrush-containing poloxamer gels. Future work will examine the effects of design parameters such as carbon nanobrush content and matrix structure on wound healing, as well as the growth of tendons and other cell lines within the hydrogel composites. This work has relevance for tissue engineering and tissue regeneration in clinical medicine.

65. A Pre-Clinical Dataset Exploring the Effects of a 19F-MRI Cellular Contrast Agent on Dendritic Cells

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The ability of cellular immunotherapy to alter a patient's immune response holds great promise for efficacious treatments in numerous disease states, including cancer. However, the mechanisms of action of these treatments are often thought to involve certain anatomical sites (i.e. lymph nodes or the tumor itself), leading to questions concerning the cellular therapeutics' dispersion and persistence, and whether or not these correlate to treatment efficacy.

In this study, we investigate the ability of a 19F MRI cellular contrast agent to elucidate alpha-type-1-polarized dendritic cell trafficking in murine models. αDC1 cells, producing high levels of interleukin-12p70 with putative strong lymph node homing capabilities, were evaluated in support of an IND incorporating post-transfer MR imaging of the therapeutic cells. DC's are co-incubated with the reagent overnight and cellular uptake is measured via 19F NMR of washed cells. To ensure the cellular behavior is not altered by the presence of label, important therapeutic functions, such as cytokine production, cellular phenotype, and antigen processing capabilities, are tested *in vitro* and compared to unlabeled control.

in vivo results demonstrate the ability of 19F MRI to detect and monitor the location of the cells post-injection. Since there is little to no native fluorine in biological tissues, any 19F signal exceeding noise threshold can be directly attributed to cellular label. Using conventional MRI, the anatomical position of the cells can be precisely determined in three dimensions, yielding the ability to calculate a quantitative measure of therapeutic cell density in known anatomical location. Using contralateral control as well as DC's dual-labeled with luciferase, details of contrast agent effects on cell viability *in vivo*, the anatomical location of cell death, and migration to the draining lymph node are examined. This dataset in its entirety provides persuasive evidence of the safety and effectiveness of cell tracking in a clinical setting.

66. Preparation of Homogeneous Progenitor Population From Human Umbilical Cord Tissue Using Gene Expression Profiling

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Mesenchymal stem cells (MSCs) from umbilical cord (UC) are regarded as the source of regenerative medicine. Isolation methods of UC-MSCs are two ways, mechanical or enzymatic dissociation method. However there were various types of cells in isolated cells from umbilical cord without application of selective isolation method. Highly homogeneous stem cells and stable quality control is an important part of cell transplantation. In this study, mononucleated cells were isolated from human

umbilical cord and analyzed cytogenetic characters of cells according passage.Population of cells was analyzed by gene expression using Illuminahuman HT-12 array in 40,000 genes. Gene expressions related with signal transduction, transport, differentiation and immune response were analyzed.About 300 genes were showed different expression in serial passage of culture expanded UC-MSCs in same donor and in passage 3 UC-MSC originating from different donor. About 1400 genes showed different expression in UC-MSCs compared to AT-MSCs. However gene expression of freshly isolated UC-MSCs was significantly different with cultured UC-MSCs because of existence of other type cells from UC (about 3200 genes). In addition, markers of mesenchymal stem cell (CD73, CD90, and CD105) were stable expressed patterns that were confirmed by FACS or RT-PCR. These data indicate that UC-MSCs after *ex vivo* expansion have high homogeneous and stable maintenance of stem cell potency. The result will be used as a standard culture of UC-MSC for preparing cell transplantation.

67. Qualification of 19F MRI to Detect CD34+ Hematopoietic Stem Cells *in vivo*

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Non-invasive detection and tracking of stem cells post-delivery lends insightful information into the mechanism of action of an intended therapy and/or possible reasons for therapeutic failure. Since the biological and physical makeup of the therapeutic cells is often quite similar to the host tissue, a chemical moiety is often needed to supply contrast or signal to some external physical method of detection. Thus, rigorous examination of the effects of the contrast agent on the cells in question must be performed to ensure minimal disruption to the therapy. In this work, we examine the effects of a novel, self-delivering 19F MRI contrast agent on hematopoietic stem cells (HSC) as well as the ability of MRI to detect cells *in vivo*.

Due to the nature of the contrast agent used, cellular labeling simply consists of co-incubation of the reagent and the cells of interest; the reagent concentration and incubation duration are parameters available for optimization of cellular viability, labeling uptake, and, if needed, cellular function post-label. Comparison of 19F-labeled HSC and unlabeled control cohorts in *in vitro* colony forming assays resulted in equal numbers of total colony forming units (CFU), as well as individual CFU types, indicating that label presence did not effect multipotency. Also, a cobblestone forming assay provided evidence that the concentration of stem-cell regenitors did not change upon labeling. *In vivo* reconstitution studies in mice, using labeled and unlabeled murine bone marrow HSC, resulted in equivalent development of CFU in the spleen, as well as reconstitution of the lymphoid and myeloid compartments. The fact that these highly complex processes remain largely unaltered in the presence of contrast agent provides strong evidence of the inertness of the reagent and that the therapeutic potential of the cells is likely maintained. Further, proof of principle MR imaging of injected and implanted HSC in a rat thigh was performed, indicating that HSC can be detected with MR methods with the use of this contrast agent. The data suggests that imaging data on the location and persistence of delivered CD34+ HSC can be obtained without influencing the function and/or differentiation potential of the therapeutic cells.

68. Regeneration of Peripheral Nerve by Newly Isolated Progenitors

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Introduction: Stem cell transplantation becomes one of the new approaches for the regeneration of peripheral nerve diseases. Previous results were demonstrated that secretion of various neural growth factors proficiently improve recovery accompanied by neural defects. The implantation of bone marrow stromal cells, adipose tissue derived stem cells, neural stem cells, and fibroblasts has been shown to improve the regeneration of peripheral nerve tissues, in this study the potential of newly isolated cells, nasal septum derived progenitor (NSDP) for the regeneration of neural defect on an 10-mm facial nerve branch lesion assessed in the rat sciatic defects.

Materials and methods: The left sciatic nerves of ten rats were transected and the tubular nerve guides were sutured to the end of the proximal and distal nerve stumps. Sciatic function index was evaluated at 2, 4 and 6 weeks after surgery using the walking track test. The control group received only the buffer solution. Six weeks after surgery, immunofluorescence staining, and histological analysis were performed.

Results: The animal which treated with cultured NSDPs showed a statistically higher number of nerve fibers, with well-shaped remyelinated axons. Also, the motor function recovery for nerve defect filled with NSDPs was faster. When compared to control NSDPs make reduced loss of gastrocnemius muscle weight. In conclusion NSDPs transplantation has been proposed as a method of improving peripheral nerve restoration.

69. Ribonucleotide Reductase-Overexpressing Pluripotent Stem Cells As a Novel Inotropic Cardiac Therapy

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Transplantation of human pluripotent stem cell-derived cardiomyocytes is a promising strategy to treat myocardial infarction and reverse heart failure, but successfully regenerating large quantities of myocardium remains a challenge. The Regnier lab has previously shown that 2-deoxyadenosine triphosphate (dATP) can replace ATP as an energy substrate for cardiac myosin and increases maximal shortening velocity, maximal force, and Ca2+ sensitivity of force. When [dATP] was increased in adult cardiomyocytes via overexpression of the enzyme ribonucleotide reductase (R1R2), contractile performance increased with no change in Ca2+ transient magnitude. Importantly, these effects were appreciated with [dATP] accounting for only 1.5% of the total cellular adenine nucleotide content, suggesting that small amounts of dATP can exert large enhance-

ments in contractility via the cooperative nature of muscle.

In preliminary *in vivo* R1R2 viral overexpression studies, we noted that cardiac performance was enhanced disproportionately to the relatively modest number of successfully transduced cardiomyocytes. ATP and other small metabolites are known to diffuse between cells via gap junctions, which suggested that dATP may be equally capable of crossing gap junctions. To test this hypothesis, we performed collaborative studies between the Regnier and Laflamme labs with the goals of (1) validating gap junction-mediated dATP transfer and (2) investigating the use of R1R2-overexpressing human embryonic stem cell-derived cardiomyocytes (hESC-CMs) as a novel therapeutic strategy for heart failure. We first performed intracellular dye transfer studies using dATP conjugated to fluorescein and demonstrated rapid gap junction-mediated transfer of fluorescence between cardiomyocytes. We then overexpressed R1R2-GFP in either hESC-CMs or fibroblasts and cocultured them with WT hESC-CMs. In both cases, WT hESC-CMs coupled to R1R2-GFP+ cells demonstrated a significant increase in contractile magnitude and velocity. Finally, we transplanted hESC-CMs overexpressing R1R2-GFP into healthy uninjured rat hearts and noted an increase in fractional shortening by echocardiography from 41±4% to 53±5% one week after transplantation. These findings suggest that transplantation of small populations of dATP-producing stem cell-derived cardiomyocytes may provide a novel strategy to enhance global cardiac function and restore contractile capabilities to pre-infarction levels without full remuscularization.

70. Robust Cardiomyocyte Differentiation From Human Pluripotent Stem Cells Via Temporal Modulation of Canonical Wnt Signaling

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Human pluripotent stem cells (hPSC) offer the potential to generate large numbers of functional cardiomyocytes from clonal and patient-specific cell sources. Here we show that temporal modulation of Wnt signaling is both essential and sufficient for efficient cardiac induction in hPSCs under defined, growth factor-free conditions. Short hairpin RNA (shRNA) knockdown of β-catenin during the initial stage of hPSC differentiation fully blocked cardiomyocyte specification while Gsk3 inhibition at this point enhanced cardiomyocyte generation. Furthermore, sequential treatment of hPSCs with Gsk3 inhibitors followed by inducible expression of β-catenin shRNA or chemical inhibitors of Wnt signaling produced a high yield of virtually pure (up to 98%) functional human cardiomyocytes from multiple hPSC lines. The robust ability to generate functional cardiomyocytes under defined, growth factor-free conditions solely by genetic or chemically-mediated manipulation of a single developmental pathway should facilitate scalable production of cardiac cells suitable for research and regenerative applications.

71. A Scalable System for Production of Functional Pancreatic Progenitors From Human Embryonic Stem Cells

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Development of a human embryonic stem cell (hESC)-based therapy for type 1 diabetes will require the translation of proof-of-principle concepts into a scalable, controlled, and regulated cell manufacturing process. We have previously demonstrated that hESC can be directed to differentiate into pancreatic progenitors that mature into functional glucose-responsive, insulin-secreting cells *in vivo*. In this study we describe hESC expansion and banking methods and a suspension-based differentiation system, which together underpin an integrated scalable manufacturing process for producing pancreatic progenitors. This system has been optimized for the CyT49 cell line. Accordingly, qualified large-scale single-cell master and working cGMP cell banks of CyT49 have been generated to provide a virtually unlimited starting resource for manufacturing. Upon thaw from these banks, we expanded CyT49 for two weeks in an adherent culture format that achieves 50-100 fold expansion per week. Undifferentiated CyT49 were then aggregated into clusters in dynamic rotational suspension culture, followed by differentiation en masse for two weeks with a 4-stage protocol. Numerous scaled differentiation runs generated reproducible and defined population compositions highly enriched for pancreatic cell lineages, as shown by examining mRNA expression at each stage of differentiation and flow cytometry of the final population. Islet-like tissue containing glucose-responsive, insulin-secreting cells was generated upon implantation into mice. By four- to five-months post-engraftment, mature neo-pancreatic tissue was sufficient to protect against streptozotocin (STZ)-induced hyperglycemia. In summary, we have developed a tractable manufacturing process for the generation of functional pancreatic progenitors from hESC on a scale amenable to clinical entry.

72. Screening 3D Stem Cell Microenvironments

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Quantitative high-content cell-based assays are currently the method of choice in the pharmaceutical and biotech industries for screening small molecule or biologic compound libraries for bioactivity and toxicity. However, few compounds identified as hits in these screening campaigns fulfill their promise at later stages in the drug development process and adverse effects which are not predicted in initial screens often result in costly failed animal trials or, worse, human clinical trials, leading to incorrectly identified hits, as well as significant unexploited

opportunities in the case of compounds whose bioactivity was missed. This high attrition rate of late stage drug candidates has led these industries to reassess the *in vitro* models used to select and validate drug targets and subsequent lead molecules. To some extent, these issues have begun to be addressed by using more relevant cell types such as primary cells or tissue-specific stem cell-derived cell lines. However, to date, most cell-based high-throughput screening approaches have largely ignored the biological context in which these cells are screened and within which the bioactive molecules are displayed. To enhance the prospects for HTS to uncover bioactive compounds which can accurately impact the complex cellular processes that underlie human physiology and disease, we have developed a 3D experimental platform which more effectively resembles the complexity and functionality of native tissues. Focusing on organotypic stem cell cultures and implementing a systems-based approach to biomaterial engineering strategies, we have developed an automated strategy for arraying cell type-specific poly(ethylene glyol) (PEG)-based synthetic microenvironments capable of supporting stem cell-based assays relevant for pharmaceutical HTS campaigns. We have shown that these arrayed 3D microenvironments support a variety of cell types, including mouse embryonic stem cells as well as breast epithelial and prostate cancer cells. Our results suggest that tight control over extracellular matrix (ECM) composition can enable the recreation of increasingly biomimetic in-vitro microenvironments, which can readily be deployed for reproducible, 3D high-throughput screening applications.

73. A Serum-Free and Xeno-Free Microcarrier-Based Scalable System for the Expansion of Human Mesenchymal Stem/Stromal Cells

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The growing need of clinical-scale numbers of human mesenchymal stem cells (MSC) for cellular therapies requires a large-scale, fully monitored and controlled bioreactor culture system for MSC production. Previous results from our laboratory have demonstrated the feasibility of MSC expansion on microcarriers using a low (2%) serum-containing medium in a spinner flask system (Eibes, G., et al. J Biotechnol. 2010). Although very promising, the use of fetal bovine serum poses a regulatory risk for cell therapy applications.

Here we report the expansion of human MSC in a microcarrier-based system using commercially available serum-free and xeno-free reagents (StemPro® MSC SFM XenoFree, Life Technologies). Firstly, spinner flask studies demonstrated the ability of xeno-free system to support expansion of MSC from bone marrow (BM MSC) and adipose tissue (ADSC) while maintaining the expected phenotype and differentiation potential. After 14 days of culture, BM MSC reached a maximum cell density of 2.0x105cells/ml (fold increase of 18), while ADSC expanded to 1.4x105 cells/ml (fold increase of 14). Then, the scale-up of this system was successfully achieved for BM MSC in a 1 L fully-controlled stirred bioreactor, reaching a cell density of 1.3±0.1x105 cells/ml (12-fold increase) after 7 days. The cells maintained tri-lineage differentiation potential and retained the MSC immunophenotypic profile. Additionally, the effect of different dissolved oxygen (DO) values on MSC proliferation and metabolism was studied: no significant differences were observed under 20% and 9% DO, while a 5% DO was shown to impair MSC expansion.

This work demonstrates the ability of a commercially available, GMP, serum-free and xeno-free medium to support a microcarrier-based, large-scale expansion of human MSC. Moreover, clinical-relevant MSC numbers required for a clinical application were obtained, after 7 days in culture, at a 1 L-bioreactor scale, representing a feasible and faster alternative to the traditional cell expansion protocol for a clinical-scale production of MSC.

74. Targeted Insertion of a Selectable Floxed Dual Fluorescence Lineage Tracing Reporter In Human Pluripotent Stem Cells by Zinc Finger Nuclease

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The conditional Cre-lox system has proven an invaluable tool for tracing cell fate decisions in developmental models (e.g. transgenic mice), but technical barriers to the genetic modification of human pluripotent stem cells have prevented its widespread application in that *in vitro* system. Homologous recombination and even stable transfection in human pluripotent cells are extremely difficult, while integrating viral vectors result in an unknown number of inserted copies and are susceptible to transgene silencing and positional effects.

METHODS: To address these issues, zinc-finger nuclease (ZFN) technology was used to stably insert a single copy of a fluorescent "stoplight" Cre-lox reporter construct into a known locus in human embryonic stem cells (hESCs). For this, a previously described floxed reporter, the mTmG transgene (Muzumdar et al Genesis 45: 593-605, 2007), was modified by the addition of a cassette for the antibiotic selection of Cre-recombined cells and flanking homology arms suitable for targeting it to the human AAVS1 locus. Gene targeting to the AAVS1 locus minimizes silencing, positional effects and off-target interference with cellular function in hESCs (Hockemeyer et al. Nat Biotechnol 27: 851-857, 2009). In cells successfully targeted with this construct (hereafter referred to as "mTmG-2a-Puro"), Cre recombinase expression permanently induces a switch from constitutive expression of the red fluorescent protein tdTomato to constitutive expression of eGFP and puromycin antibiotic resistance.

RESULTS: The mTmG-2a-Puro reporter was successfully targeted to the AAVS1 locus in undifferentiated RUES2 hESCs, with site-specific integration verified by Southern blot. To assess the kinetics of the fluorescence transition after Cre expression, we transduced the resultant mTmG-2a-Puro hESCs with a lentivirus expressing Cre under the constitutive EF1α promoter and tracked the percentage of red and/or green fluorescent hESCs by daily flow cytometry. eGFP+ cells were first

detected at 24 hours and became a distinct population from tdTomato+ cells over several days. The eGFP+ cells were isolated by treating the mixed population with puromycin, resulting in a nearly 100% eGFP+ tdTomato- population. To test the function of our system in differentiated hESC progeny, we transduced differentiated cultures (containing ~60% cardiomyocytes) with a lentiviral vector that encodes Cre recombinase under the control of the striated muscle specific MCK-CK7 promoter. eGFP+ α-actinin+cardiomyocytes were then selected with puromycin to greater than 98% purity.

CONCLUSION: We have successfully created a novel undifferentiated hESC line that contains a convenient "stoplight" fluorescence reporter suitable for Cre-lox based fate mapping studies. This system should be useful in the purification of any cell population that has been indelibly marked by Cre recombinase expression.

75. Temporal Application of Topography to Increase the Rate of Neural Differentiation From Human Embryonic Stem Cells

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Human embryonic stem cells (hESCs) have great potential for neurological repair and regeneration; but first, the mechanisms governing neural differentiation must be understood and applied for increased efficiency. Several groups have generated a variety of neural differentiation protocols, and these protocols have been further improved upon and/or adapted for additional hESC lines. These protocols have predominantly focused on using various biochemical cues in a temporal manner; however, one study explored the use of topographical cues to differentiate hESCs into neurons. Lee et al. cultured hESCs on 350nm gratings in serum-containing media for 24 hours, followed by media with serum replacement for an additional 5-10 days. They concluded that neuronal differentiation was improved when cultured on 350nm gratings. However, it remains unclear how topographical cues affect neuronal differentiation. In this study, we modified the protocol by Wu et al. to quantitatively study the topographical effect of 2μm gratings on hESC neural differentiation. We found that 2μm gratings increased the rate of differentiation to neural progenitor cells as indicated by an increase in gene and protein expression after an initial 7 days of exposure to the topography without additional biochemical cues. The differentiation continued to progress at an increased rate after a second exposure to the topography and additional biochemical cues. We anticipate that this work will allow us to elucidate the temporal importance of topography on neural differentiation of pluripotent stem cells; and thus provide the field with a more cost effective and efficient method of generating hESC-derived neural cells.

76. Three-Dimensional Culture and Differentiation of Human Adipose Tissue-Derived Stromal Cells In Chitosan/α-Glycerophosphate/Collagen Hydrogels

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in vitro culture and *in vivo* differentiation of human adipose tissue-derived stromal cells (ADSCs) in chitosan/β-glycerophosphate/collagen (C/GP/CO) hydrogel were studied. The ADSCs were harvested and mixed with C/GP/CO hydrogel followed by gelation at 37°C and *in vitro* culture. Cells' expansion,viability and morphology were detected. The biocompatibility of the hydrogel was investigated by subcutaneous injection into SD-rats. Adipose-induced ADSCs was mixed with C/GP/CO hydrogel and subcutaneously injected into nude mouse and the adipogenic differentiation *in vivo* was observed. The results showed that ADSCs cultured in C/GP/CO hydrogel expanded by 30% within 7 days. Cells were well adhering to the hydrogel with good morphology and high viability after 5 days of *in vitro* culture. Immunostaining analysis of the implants and surrounding tissue showed that the C/GP/CO hydrogel had good biocompatibility within 4 weeks. Staining results of cell-hydrogel complex implants after 4 weeks of culture *in vivo* indicated that a great number of adipocytes were formed with vascularization while the results of complex implants with non-induced ADSCs were negative. This work was supported by DUT11SM09, the NSFC30700181/31170945 and The Project-sponsored by SRF for ROCS, SEM.

77. Three-Dimensional Culture of Neural STEM/Progenitor CELLS/COLLAGEN Sponge Constructs IN Perfusion Bioreactor

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BACKGROUND: Neural-like tissues can be fabricated by three-dimensional (3-D) culture of neural stem/progenitor cells (NS/PCs) *in vitro*. When 3-D construct approaches a certain scale, however, the nutrient supply becomes limited in static culture. A perfusion bioreactor was designed for cells-scaffolds and the results were compared with those of static culture in this study.

METHODS: Collagen sponges, as the 3-D scaffold were immersed in the culture medium with 5 μg/ml laminin before seeding. Then, the cell-scaffolds constructs were transferred into the bioreactor and 24-well plate as the static control. Cells number was calculated with haemacytometer and the NS/PCs morphology and cells distribution in collagen sponges were detected by immunofluorescence staining and HE staining under inverted microscope. Meanwhile the glucose, lactate concentrations and pH of the two different culture methods were measured by biochemical analyzer and PH meter.

RESULTS: The proliferating (nestin+) NS/PCs were distributed more uniformly in the collagen sponge scaffolds in bioreactor than in static culture. And the amplification multiple of NS/PCs cultured under dynamic conditions was also significantly higher than static conditions. The concentration of glucose in bioreactor remained a stable value, while the lactate stayed in a lower level, so the PH value maintained at normal physiological range during the culture process in bioreactor. It can be concluded that laminin pretreated collagen sponges can promote NS/PCs adhesion. And perfusion

bioreactor was benefit for NS/PCs proliferation since a stable physical environment was provided by a dynamic system. This work was supported by DUT11SM09, the NSFC30700181/ 31170945 and The Project-sponsored by SRF for ROCS, SEM.

78. Two Human Embryonic Stem Cell Derived Clonal MSC Cell Lines Showed Distinct Proliferation and Differentiation Properties

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Human embryonic stem cells (hESC) have the potential to generate virtually any differentiated progenies that are an attractive cell source for transplantation therapy, regenerative medicine and tissue engineering. To realize this potential, it is essential to be able to control ESC differentiation and to direct the development of these cells along specific pathways. Several protocols have been reported in which hESCs were directed to differentiate into musculoskeletal tissues.

We have shown in a previous study that by inhibited the transforming growth factor β (TGF- β) signaling pathway during human ESC differentiation as hEBs using SB-431542 (SB) in serum-free medium lead to selective up-regulation of several markers involved in mesoderm and myogenic differentiation. Explant cultures of hEBs in presence of 10 % serum stimulated hESC derived MSC differentiation of SB-OG cells. The hESC derived MSCs (hESC-MSC) expressed all known MSC cell surface markers and had a very similar gene expression profile to bone marrow derived MSCs. *in vitro* they differentiated into osteoblasts and adipocytes as primary MSCs, and *in vivo* we observed cartilage and ectopic bone formation. Recently, we isolated two clonal cell lines from these hESC-MSCs entitled T1 and T3, both cell lines showed initially identical cell surface profile. The CD markers changed their expression profile in T1 during successful passaging the cells. T1 showed lower cell population doubling time and lost the expression of two MSC markers: CD90 and CD146, whereas T3 maintained high proliferation and no change in expression of all CD markers. On the other hand T1 showed very high osteoblastic and adipogenic differentiation potential, contrary to T3 which had very low differentiation frequency. Both cell lines have been extensively characterized by FACS, qPCR, and Transmission Electron Microscopy. Both cell lines have been stimulated with different growth factors to stimulate proliferation in T1 and differentiation in T3 to understand the molecular mechanism behind this difference in behavior.

We consider that the clonal cell line T1 is a more differentiated osteo/adipo-progenitor cell line which thus easily differentiates into osteoblasts and adipocytes. On the contrary, T3 is possible a more stem cell like population which needs growth factor stimulation for proper differentiation. Access to lineage-specific progenitors for transplantation will allow comparison to more mature populations to determine which stage integrates best into the adult tissue and which ultimately provides the most benefit. With these tools at hand, the therapeutic potential of hESCs is now ready to be tested for example in the field of mesenchymal lineages.

79. Using Electrospun Poly (e-caprolactone) Nanofibers to Promote the Differentiation of Induced Pluripotent Stem Cells Into Neural Phenotypes

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The aim of this project is to enhance the differentiation of induced pluripotent stem cells (iPS) into neural phenotypes through using biocompatible and biodegradable non-woven electrospun scaffolds. In particular, we anticipated the high surface area and three dimensional aligned topography of electrospun poly (e-caprolactone) (PCL) nanofibers would promote proliferation and differentiation of iPS-derived neural progenitors into neurons. An electrospinning set-up consisting of a syringe pump, a nozzle machined Teflon, a water container for nonalignment fibers, drum fiber-collector for alignment fibers, and a high voltage power supply was used to fabricate the PCL nanofibers. A mixture organic solvent of chloroform and methanol with the volume ration of 7:1 was prepared to dissolve 10 % PCL (w/v). PCL solution was stirred overnight at room temperature at 1100 rpm. Afterward, the PCL solution was pumped at the constant flow rate of 2ml/hr to electrospinning syringe. Both random and aligned electrospun PCL nanofibers were transferred to loading stubs to characterize the morphology through scanning electron microscopy (SEM). Additionally, PCL fibers were loaded with retinoic acid (RA) using a blending technique to further enhance the differentiation of iPS to neurons. IPS cells were induced to form embryoid bodies in suspension and treated with a combination of retinoic acid and purmorphamine to promote differentiation into neural lineages. These embryoid bodies were then seeded upon sterile electrospun scaffolds. . The resulting cultures were analyzed using immunohistochemistry to assess neuronal differentiation. Scaffolds consisting of aligned electrospun PCL and PCL/RA fibers could promote iPS cell differentiation into the desired phenotypes through it nanostructures cues and sustained release of chemical cues. The combination of the fibrous scaffolds and iPS could be used as a strategy for spinal cord injury repair.

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