

2009 ISSCR Industry Wednesday Symposia

- **Symposium title**

New Tools and Technologies to Accelerate Stem Cell Research

- **Name of supporting company**

Thermo Fisher Scientific

- **Statement of educational need and/or benefit for holding the symposia**

With the rapid advances taking place in stem cell research, it is valuable for researchers to stay abreast of the latest tools and technologies that are available to advance their understanding of stem cells. Attendees of this symposium will learn about a range of technologies that span the stem cell work flow, from new iPS kits and culturing surfaces, to current methods for gene modulation, cellular imaging and analysis and protein expression. The presentations will illustrate how these tools are being applied to advance the field of stem cell biology.

- **Narrative description of the symposium**

Discoveries in stem cell biology continue to advance at a rapid rate. The use of stem cells as tools for basic research and increasingly, for clinical therapies, is reported almost daily. Yet many challenges remain. This symposium will help researchers gain a better understanding of some of the newest tools and technologies they can employ to enhance their studies, including the efficient production of iPS cells; the use of stem cell progenitors to accelerate toxicology screens; the newest siRNA technology and a highly efficient transfection method that facilitates the study of gene function in neural stem cells; and the discussion of a novel surface that allows non-enzymatic subculture of stem cells and facilitates stem cell research and tissue engineering studies. This symposium will also discuss the use of proteomic technologies and high-content image analysis techniques as applied to the study of stem cell differentiation and the identification of genes and proteins involved in stem cell growth and differentiation.

- **Learning objectives and goals of the symposium:**

Symposia attendees will learn about:

- The efficient production of iPS cells via a complete iPSC kit
- The use of stem cell progenitors to accelerate toxicology screens
- A novel surface that allows non-enzymatic subculture of stem cells and facilitates stem cell research and tissue engineering studies

- The newest siRNA technology and a highly efficient transfection method that facilitates the study of gene function in neural stem cells
- High-content data acquisition methods for quantitatively detecting up-regulated and down-regulated proteins that are involved in major lineage decisions
- The use of proteomic technologies and high-content image analysis as applied to the study of stem cell differentiation and the identification of the genes and proteins involved in stem cell growth and differentiation

1. Faster and easier research using rapid iPSC cell kits and progenitor cell lines

Steven Stice, PhD, Professor, Director Regenerative Bioscience Center, GRA Eminent Scholar, University of Georgia and CSO, Aruna Biomedical, Inc.

ArunA Biomedical and Thermo Fisher have developed a complete human induced pluripotent stem cell kit that significantly reduces the amount of time required to generate iPSC colonies. We produced colonies large enough to passage and propagate to 13- 20 days post transduction using 6 reprogramming factors. Most reprogramming protocols require 20 to 30 days. IMR-90 human lung fibroblasts transduced with the 6 factors achieved a 0.03-0.04% reprogramming efficiency, which is consistent with previous studies, and these pluripotent cells have in vitro characteristics similar to human embryonic stem cell cells.

ArunA biomedical has also developed technology to direct pluripotent stem cell differentiation into various progenitor cell types. Our serum-free and feeder-free adherent monolayer technology platform provides “off the shelf” access to normal human progenitors. Aruna’s neural progenitor cells have the potential of turning into a wide variety - if not all-cell types of the central nervous systems. The cells are robust, easily maintained, and highly proliferative. This cellular technology, combined with Aruna’s proprietary qualified media and plate coating systems, make their stem cell kits an efficient and economical choice for drug discovery and basic research.

Dr. Steven Stice, PhD

Professor
Director, Regenerative Bioscience Center
GRA Eminent Scholar
CSO Aruna Biomedical Inc.
University of Georgia
Athens, GA

Biographical Summary:

Dr. Steven Stice is CSO of Aruna Biomedical Inc. and a Professor and Director of the Regenerative Bioscience Center and has a Georgia Research Alliance Eminent Scholar

endowed chair. Prior to joining the University of Georgia, Dr. Stice was a cofounder and Chief Scientific Officer at Advanced Cell Technology, a stem cell and cloning company. Throughout his career he has published and lectured internationally on the topics of cloning and stem cells. In 2001, three of the human embryonic stem cell lines that Dr Stice's lab derived were approved for federal funding by President Bush. In 2006, Dr. Stice founded Aruna Biomedical, Inc., the first group to market a product derived from human embryonic stem cells (2007). The product is a neural stem cell used for research on neurological diseases and disorders, ranging from Parkinson's disease to depression.

2. Temperature-Responsive UpCell Surface Enables Enzyme-Free Harvesting of Single Cells and Tissue Engineering Without Scaffolds

Thomas Brevig MD, PhD, Director Global Research, Thermo Fisher Scientific

Anchorage-dependent mammalian cells attach initially to proteins adsorbed to the cultureware from the medium. During the course of cultivation, the cells deposit extracellular matrix (ECM) molecules on the surface of the cultureware. Human endothelial cells, for example, deposit collagen type IV, the main structural component of the basal lamina. Whereas dissociation of cells from the cultureware is traditionally done by enzymatic or mechanical methods our novel surface enables dissociation of cells from the cultureware upon a simple change in temperature. Being slightly hydrophobic at 37°C, the Nunc UpCell surface allows cells to attach and grow, but hydrophilic when the temperature is reduced to below 32°C, the UpCell surface binds water and swells, resulting in the release of adherent cells with their underlying ECM intact. This non-enzymatic cell harvesting method preserves (1) cell membrane proteins, which facilitates analysis of, and enrichment procedures based on, cell-surface markers; and (2) ECM molecules, enabling harvesting of contiguous cell sheets, and creation of tissue models and transplants held together only by normal cell junctions and cell-deposited ECM.

Single cells may be harvested from a sub-confluent culture by temperature reduction while maintaining cell viability and the integrity of surface receptors and antigens. For example, the integrity of CD140a was better preserved on human bone marrow cells and preadipocytes harvested from the UpCell surface by temperature reduction than when these cells were harvested from traditional cultureware using a short trypsinization. Even cell types that are difficult to detach by other methods can be harvested from the UpCell surface. The recovery of mouse peritoneal macrophages from the UpCell surface was significantly higher than the recovery of these cells from traditional cultureware using trypsinization or scraping.

Contiguous cell sheets with preserved cell polarization and cell-cell junctions may be harvested from a confluent culture using a supporting membrane and temperature reduction. The retention of the ECM enables the attachment of one cell sheet to another cell sheet or a graft site without the use of fibrin glue or sutures. Tissue models, co-cultures and grafts can thus be created without scaffolds and exogenous materials,

allowing control of the spatial distribution of cells in 3D, while minimizing the risk of host inflammatory reactions and fibrous tissue formation. In transplantation models, single sheets may be grafted or multi-sheet constructs may be used where sheets can be stacked prior to or during the transplantation procedure.

Thomas Brevig MD, PhD

Director Global Research
Nunc and Nalgene
Thermo Fisher Scientific

Biographical Summary:

Dr. Thomas Brevig is Director, Global Research for Nunc and Nalgene products, Thermo Fisher Scientific. He graduated in Medicine in 1998 and received his PhD in Neuroscience in 2001, both from University of Southern Denmark. He has made scholarly contributions in the fields of transplantation immunology, brain repair, biomaterials and cell culture and analysis.

3. Efficient Gene Knockdown in Human Neural Stem Cells Using Thermo Scientific Dharmacon Accell siRNA

Stephanie Urschel^{1}, Jennie N Jeyapalan², Vanessa J Appleby², Dzul Azri Mohamed Noor², Shih-Han Lee² and Paul J Scotting²*

Gene knockdown by RNA Interference (RNAi) is recognized as a valuable technology for identification of pathways that contribute to cell fate determination. Unfortunately, common siRNA delivery techniques such as electroporation and lipid-mediated delivery are ineffective and/or toxic in some cell types, thus demanding new technologies for delivery of these gene silencing reagents. In this talk, we will discuss the newest generation of siRNAs, Thermo Scientific Dharmacon Accell siRNA, which can be delivered to cells without the use of lipids or electroporation. Results of detailed studies regarding the development and testing of Accell siRNA (including the efficiency of delivery in different cell types, toxicity studies, innate immune responses, and off-target effects) will be presented together with examples of applications of Accell technology in human neural stem cells.

*Thermo Fisher Scientific¹, Children's Brain Tumour Research Centre, Institute of Genetics, School of Biology, QMC, University of Nottingham², Nottingham, UK, NG7 2UH; * lead contact*

Stephanie Urschel, PhD

Sr. Field Application Scientist

Dharmacon and Pierce/Endogen
Thermo Fisher Scientific

Biographical Summary:

Dr. Stephanie Urschel received her doctoral degree in chemistry from the University of Bonn, Germany in 2001. Her thesis focused on the regulatory mechanisms for gap junction channel activity applying protein biochemical and molecular genetic methods. She performed her postdoctoral work from 2001 to 2005 in the lab of Prof. Klaus Willecke (Department of Molecular Genetics in Bonn, Germany) on the biochemical analysis of transgenic mouse models. In 2005 she joined Perbio Science as Scientific support Specialist for Dharmacon and Pierce/Endogen. Since April 2006, she has held a position with Thermo Fisher Scientific as European Field Application Scientist for the Thermo Scientific Dharmacon products.

4. A High Content Imaging Approach to Stem Cell Research

Katrina Drayton, Field Application Scientist, Thermo Fisher Scientific

Understanding the signaling processes, differentiation and physiology of stem cells is key to their use and this often involves painstaking, manual experiments. High content imaging offers the capability to make multiple quantitative cell-by-cell measurements, in a rapid and automated fashion, coupled with the ability to specifically define and analyze targeted cellular populations. This technology is already being applied to understand stem cell biology at the cell level and has the potential to accelerate this important area of biology, through deeper insights and greater experimental scalability. This talk will review the advantages of high content imaging technology, discuss its current application to stem cells and provide three case studies on its use.

Katrina Drayton

Field Application Scientist
Cellular Imaging and Analysis
Thermo Fisher Scientific

Biographical Summary:

Katrina Drayton graduated from University College London in Biochemistry and Molecular Biology in 1991. She began her professional career with Fisons/AstraZeneca Charnwood as a Senior Research Scientist, where she spent 13 years developing and running robust cell-based and biochemical assays, including automated assay platforms used in UHTS for AstraZeneca's lead generation unit. In this role she acquired

experience with a wide variety of assay formats and technologies, including, FLIPR, FMAT, AlphaScreen and ECL. In 2004 Katrina joined Cellomics (now part of Thermo Fisher Scientific) as a Field Application Scientist, and over the past 5 years has used her expertise to support Thermo Fisher customers across Europe in both academic and commercial settings with broad and varied High Content Analysis project requirements.

5. Protein Expression Profiling of Stem Cells with SILAC and Tandem Mass Tag (TMT) Reagents

Michael M. Rosenblatt, PhD, Sr. Research Scientist, Thermo Fisher Scientific

To identify protein expression changes during stem cell differentiation, we have used complementary protein profiling strategies by mass spectrometry in two biological systems. In the first model, high content imaging analysis, in cell ELISA, and SILAC technologies were used to evaluate differentiation and to study the protein expression changes in adult adipose derived mesenchymal stem cells during neuronal differentiation. An alternative strategy using phosphoproteome enrichment and Tandem Mass Tags was used to study quantitative phosphoproteomic changes in glioma cancer stem cells using in response to hypoxia, inflammatory signals and STAT3 inhibition. Protein profiling of stem cells offers great promise and valuable insights into cellular differentiation and cancer development.

Michael M. Rosenblatt, PhD

Sr. Research Scientist

Life Sciences/Mass Spectrometry Reagents

Thermo Fisher Scientific

Biographical Summary:

Michael M. Rosenblatt received his undergraduate degree (cum laude) from Towson University in 1992. He received his PhD degree from the University of Illinois in the area of Biological Inorganic Chemistry in 2000. He then became an NIH Post-Doctoral Fellow in the Laboratory of William F. DeGrado at the University of Pennsylvania School of Medicine until 2003. From 2003 – 2007 he was the founding director of the Proteomics laboratory at the Children's Hospital of Philadelphia. In July of 2007, he joined the Life Sciences group of Thermo Fisher Scientific in Rockford, IL where he works as a Senior Research Scientist in the Mass Spectrometry Reagents group.