

# 2017 Medicine by Design Symposium Report

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## Abstract

The second annual Medicine by Design Symposium was held at the MaRS Discovery District in Toronto on December 5, 2017. Researchers and trainees from the University of Toronto (U of T) and its affiliated hospitals, and representatives from the health-care sector, industry, business and government attended the symposium.

Talks by Medicine by Design principal investigators addressed four key areas:

1. enabling technologies and developmental models;
2. immunoengineering;
3. organoids and tissue models; and
4. tissue and organ repair.

Donald Ingber, founding director of the Wyss Institute for Biologically Inspired Engineering at Harvard University, gave the keynote address. Other highlights included research on immunoengineering presented by Darrell Irvine, a professor of materials science and engineering and bioengineering at the Massachusetts Institute of Technology (MIT) and a member of Medicine by Design's Scientific Advisory Board, and a trainee poster session featuring more than 50 posters.

## Theme 1: Enabling Technologies and Developmental Models

Numerous challenges must be overcome to successfully apply regenerative medicine approaches to the treatment of chronic conditions. In the first session of the day, researchers presented their work to develop new technologies and developmental models that will allow us to address many of these challenges with a focus on cell manufacturing technologies, tools for evaluating the essentiality and fitness of genes expressed by stem cells, and a drosophila model to investigate how contractile forces affect wound healing in the embryonic heart.

### New Technologies for Stem Cell Isolation and Analysis — Shana Kelley (Leslie Dan Faculty of Pharmacy, U of T)

Stem cells remain critical to the development of promising regenerative medicine-based approaches to treat and study disease. Hurdles exist, however, in efficiently purifying, expanding and analyzing stem cells. Shana Kelley's lab has developed a number of innovative tools to overcome these challenges. These tools include sensors that directly measure specific factor concentrations in the culture medium for process control of stem cell bioprocesses, gene circuits for multiplexed analyte panels, and a microfluidics-based system for the isolation of stem cells.

In collaboration with Peter Zandstra (Institute of Biomaterials & Biomedical Engineering (IBBME) at U of T, and School of Biomedical Engineering, UBC), Professor Kelley has developed electrochemical sensors for biochemical analysis that exhibit a high level of sensitivity and specificity. These electrochemical sensors are being used to monitor the concentration of secreted inhibitory factors in hematopoietic stem cell cultures with the goal of developing smart fed-batch cultures that enable real-time monitoring and control of these inhibitory factor concentration to optimize hematopoietic stem cell yield.

Professor Kelley's work extends to developing next generation electrochemical reporters to enable automated monitoring and analysis of cells directly in culture. This reporter system is based on gene circuits comprised of pieces of DNA that respond to analyte inputs by expressing a specific protein that can be easily detected. The development of electrochemical reporters allows for more efficient and accurate monitoring of cells in culture.

Conventional methods of isolating cells can be laborious and time consuming. This is especially true when isolating stem cells. Professor Kelley has developed a method for high-resolution separation of cells using nanoparticles. Coated magnetic nanoparticles bind to specific proteins known to be expressed on the membrane of the cells of interest. Nanoparticle-bound cells are separated and sorted based on the destructive magnetic ranking of cytometry (the number of nanoparticles on a cell's surface will dictate whether it will stick to the wall of the cytometer). This method is similar to fluorescence-activated cell sorting (FACS) but has the advantage of being faster (1 hour versus 12 hours with FACS) and higher-throughput, resulting in higher cell survival rates. With this sorting system, in a collaboration with Derek Van der Kooy (Department of Medical Genetics) the Kelley Lab was able to enrich the stem cells from 1:1000 cells (ratio of stem cells to non-stem cells found in the primary dissection of ciliary epithelium) to 1:2 (or 50% of the cells isolated gave rise to clonal retinal stem cell derived spheres). This is an incredible achievement since, in vivo, retinal stem cells can not be identified prospectively. Enriching the stem cell pool enables identification of its molecular signature which can then be used to both purify the population in vitro and identify this population in vivo.

### Probing the Essentialome in Stem Cells — Jason Moffat (Donnelly Centre for Cellular & Biomolecular Research, U of T)

Essential genes are those that are indispensable and required for cell survival. In studying diseases, Jason Moffat found that the number of pathological mutations of a gene correlated with its essentiality. His lab generated a Toronto knockout library that can be applied across multiple cell types to identify genes that drive pathogenic cell types.

In addition, Professor Moffat and his team conducted a mini genome-wide CRISPR screen to identify fitness genes that are expressed in a context-dependent manner in adherent and suspension cells. The mechanism for context-dependent fitness gene expression involves substrate-dependent sensitizing and masking of genetic interactions. Results of the genome-wide screen identified that expression of the Integrin AV gene is essential for the adherence of cells to a substrate. In suspension culture of spherical cell aggregates formed with Integrin AV knockout cells, cell detachment was observed on the aggregate surface. Notably, a number of stem cell marker genes were turned on in Integrin AV knockout cells indicating that this gene is involved in regulating stem cell maintenance.

### Embryonic Wound Repair: Mechanochemical Signalling at the Leading Edge —

Rodrigo Fernandez-Gonzalez (Institute of Biomaterials & Biomedical Engineering, U of T)

Following myocardial infarction, the healing process results in scarring which manifests as a stiffening of the tissue at the injury site. This stiffened tissue can impede the normal beating of the heart which ultimately leads to heart failure. Rodrigo Fernandez-Gonzalez and his team are exploring this process in the *Drosophila* embryo where wound healing occurs in the absence of scarring. Embryonic wound repair is driven by the rearrangement of actin-myosin, a contractile protein. The *Drosophila* Abelson (Abl) tyrosine kinase mutant has defective actin organization, which results in slower wound closure. A key observation made by the Fernandez-Gonzalez lab was that in wild-type embryos contractile force varies along the wound edge whereas in Abl mutant embryos (with perturbed actin-myosin contractile protein organization) it does not. This finding led them to examine the role of contractile force heterogeneity in facilitating wound closure. Using mathematical modeling to represent all cells *in silico*, they found that strain drives myosin recruitment and dynamics, contributing to faster wound closure. This observation challenges the conventional model of wound healing in which wound closure is thought of as a “purse string” with uniform contractile forces driving closure.

## Theme 2: Immunoengineering

In the second session of the symposium, researchers discussed engineering immune cells that target cancer and challenges in advancing promising findings towards commercial cell therapies in Canada.

### Engineering Approaches for Enhanced Cancer Immunotherapy — Darrell Irvine

(Department of Biological Engineering, MIT)

Adoptive cell therapy is a promising approach to treat hematopoietic cancers, such as leukemia, however has shown limited effectiveness in the treatment of solid cancers. Darrell Irvine discussed “hitching” therapeutics onto lymphocytes as a way of improving adoptive cell therapy. In this technique, nanoparticles carrying therapeutic small molecules are stably linked to lymphocytes (e.g. T-cells) through the thiol groups present on cell surface proteins. While free particles do not accumulate in a tumour, particles attached to T-cells do. Professor Irvine stated that this technique could be used to increase the efficacy of chemotherapy. However, two challenges remain: the loading capacity of the drugs, and effectively transporting the drugs to the tumor location. To address these challenges, Professor Irvine developed a “protein nano-gel” that would un-crosslink when it reaches a tumour. Given that activated T-cells have a higher rate of redox than non-activated T-cells, protein nano-gels were created with thiol bonds that cleave during cell surface redox. These T-cells with drugs “backpacked” in protein nano-gels more effectively deliver drugs to solid tumors and have lower toxicity compared to systemically delivered drugs.

### Designing T-Cell Lymphocytes — Juan Carlos Zúñiga-Pflücker (Sunnybrook Research Institute, Sunnybrook Health Sciences Centre)

After chemotherapy and stem cell transplantation, leukemia patients’ experience deficient T cell production which typically lasts for 6 to 12 months and leads to several complications including opportunistic new infections, reactivation of latent viral and fungal infections, and failure to thrive. Juan Carlos Zúñiga-Pflücker discussed co-transplanting T-cells derived from patient blood stem cells as an immunotherapy for leukemia patients. He emphasized that the first step

to establishing this therapy is understanding the early events in the thymus that instruct blood stem cells to become T-cells. His lab developed an in vitro co-culture system using OP9 feeder cells that secrete Notch ligand to blood stem cells, mimicking the environment in the thymus where T-cells develop. Using this system, Professor Zúñiga-Pflücker has successfully generated human T-cells from blood stem cells. Importantly, his lab has functionally validated these human T-cells in vivo in a mouse model, demonstrating efficient engraftment in the thymus which led to enhanced T-cell reconstitution in the bone marrow. Next, Professor Zúñiga-Pflücker will pursue new clinically- and commercially- relevant approaches to produce T-cells for therapeutic applications, focusing on animal cell-free Notch delivery methods that are amenable to scalable production in suspension.

### A Solution for Cell Therapy Safety: Paving the Way to the Clinic — Andras Nagy (Lunenfeld-Tanenbam Research Institute, Sinai Health System)

Andras Nagy described his vision of having one safe cell line to treat all of humankind. He proposed learning from the Klingons' (a fictional extra-terrestrial warrior species from *Star Trek*) technology to “cloak” donor cells from the host immune system. Eight cloaking transgenes were identified and used to create a cell line (NT2). When these cells were transplanted into multiple mouse strains, teratomas formed showing that cells were properly cloaked. However, the appearance of these tumors also presents the downside of hiding cells from the immune system - the development of cancer in the patient. To overcome this, Professor Nagy discussed the creation of fail-safe cells with absolute external controls on cell proliferation. These fail-safe cells contain two suicide genes that kill all proliferating cells. Professor Nagy also ensured the cell lines were homozygous as they previously observed that loss of heterozygosity led cells to lose suicide genes. Finally, the immuno-cloaking and fail-safe systems were combined to develop a cell line that generated stable teratomas in a variety of mouse strains.

### PANEL: Planning for a Successful Pathway to Adoption

The second session ended with a panel discussion, moderated by Patrick Bedford, senior manager, clinical translation and regulatory affairs at CCRM, on how to successfully bring regenerative medicine therapies into practice. Dion Neame from Sanofi Pasteur emphasized the difficulty in getting people to pay for therapy after regulatory approval. He also stated the need to translate knowledge to the government through evidence, economics, patient advocacy and media. Shahira Bhimani discussed MaRS EXCITE, an initiative with the goal to foster the adoption of innovative technology. She stated that EXCITE helps young companies that do not have the resources to bring a product to market on their own. EXCITE selects technologies that are anticipated to have the greatest impact and creates a roadmap to move technologies into practice. Finally, Shirlee Sharkey, president and CEO of Saint Elizabeth Health Care, spoke about the importance of innovation in society.

### Theme 3: Organoids and Tissue Models

The third session focused on novel technologies for modelling biological events using organoids and tissue models. It featured Donald Ingber, the keynote speaker for the symposium, who highlighted his organ-on-a-chip work.

### Catalyzing the Next Technology Wave: Biologically Inspired Engineering at the Wyss Institute — Donald Ingber (Wyss Institute for Biologically Inspired Engineering, Harvard University)

As director of the Wyss Institute, Donald Ingber focuses on developing biomimetic microsystems to explore biological questions. Dr. Ingber explained that he believed the current drug development model was broken, and pre-clinical results (*in vitro* or *in vivo*) often did not predict clinical outcomes. He hypothesized that many *in vitro* platforms fail to incorporate biologically relevant mechanotransduction cues in their designs, which motivated him to develop his lung-on-a-chip model.

The microfluidic chip used in his model was designed with a tunable vacuum chamber to mimic a mechanically active lung structure. Using their lung-on-a-chip model, Dr. Ingber's group was able to assess the dynamics of different cytokines and found that their model produced comparable results to observations made *in vivo*. Dr. Ingber hypothesized that his microfluidic chip platform could potentially offer an economical and practical replacement to conventional animal models. In addition, this mechano-active microfluidic chip could be adapted to model different organs, such as the heart or small intestine. His team is currently working on commercializing these organs-on-chips, with the goal of designing personalized organs-on-chips, which could reduce the need for clinical trials.

### Modelling the Adult Human Neuromuscular Junction in a Dish — Penney Gilbert (Institute of Biomaterials and Biomedical Engineering, U of T)

Many different skeletal muscle diseases are caused by problems in the neuromuscular junctions. To study these neuromuscular junctions *in vitro*, Penney Gilbert's lab successfully developed a 3D muscle stem cell culture system to model adult neuromuscular junctions representative of those found *in vivo*. Using this model, Professor Gilbert's lab has been able to measure motor neuron clusters and neurotransmitter release in response to stimuli. The reliable measurement of several neuromuscular junction parameters enabled by this platform, has allowed Professor Gilbert's to assess changes that occur during the progression of a number of different neuromuscular diseases, such as amyotrophic lateral sclerosis.

### SPIM-ing the Light Fantastic: Platforms for Imaging Tissues and Organoids — Christopher Yip (Institute of Biomaterials and Biomedical Engineering, U of T)

Christopher Yip started his talk with his lab's science and technology mantra which he referred to as the 4F's: flexible, fast, focused and free. His lab focuses on engineering combinatorial microscopy to acquire real-time spatial information to observe the kinetics of molecular self-assembly. SPIM stands for Selective Plane Illumination microscopy (light sheet), a technique used to rapidly image large tissues and organoids. The advantage of light sheet microscopy over conventional confocal microscopy is that it focuses perpendicular to the plane rather than focusing through the plane. Using their home-built SPIM microscope, Professor Yip's lab can image tissue organoids without worrying about photo-bleaching.

### Engineering Functional Human Islets from Embryonic Stem Cells — Cristina Nostro (Toronto General Hospital Research Institute, University Health Network)

Type I diabetes is a disease in which insulin-producing beta cells are destroyed and glucose homeostasis is disrupted. Pancreatic islet transplantation may eliminate the need for insulin

injections to control blood glucose levels in diabetes patients, however multiple donors are required for a single transplantation limiting the effectiveness of this therapy. Cristina Nostro's lab is focused on understanding the mechanisms that govern the differentiation of human embryonic stem cells (ESC) to pancreatic islets with the goal of producing an alternative source of pancreatic islets. The main obstacle to generating in vitro ESC-derived pancreatic islets has been variability in differentiation efficiency. However, Professor Nostro's group has made the exciting discovery GP-2, a cell surface marker, is specifically expressed by pancreatic progenitor cells. Using this new cell surface marker to isolate GP-2+ pancreatic progenitor cells, it was found that these cells differentiate more efficiently into insulin-producing cells than the unsorted population. Her lab continues to probe different markers and pathways to increase the efficiency of pancreatic cell differentiation, with the hopes of generating a reliable alternative source of insulin-producing cells for transplantation.

## Theme 4: Tissue and Organ Repair

The fourth session in the symposium focused on the lung, as a model organ to demonstrate how regenerative medicine be applied to address the issue of organ shortages as a limitation in organ transplantations.

### Decellularization and Recellularization for Lung and Airway Regeneration — Thomas Waddell (Division of Thoracic Surgery, University Health Network)

Organ donor shortage remains the major limitation to performing lung transplantations. An exciting method to overcome this issue is the recellularization of decellularized lungs. However, this requires appropriate vascularization of the decellularized lung. A potential source of endothelial cells for lung vascular engineering is induced pluripotent stem cells (iPSCs). The Waddell lab has proposed using partially reprogrammed cells known as induced progenitor-like cells (iPLCs). When cultured on decellularized lung in a reperfusion bioreactor, these cells start to express endothelial cell markers. Moreover, organ specificity was not obvious after recellularization. This suggests that partial reprogramming is an efficient method to get a specific phenotype without the need for extensive differentiation protocols. Moving forward, the Waddell lab is investigating how to denude the epithelium and reseed to avoid transplant rejection.

### Promoting Recovery of the Stroke Injured Brain: Cells, Drugs and Neural Repair — Cindi Morshead (Division of Anatomy, Department of Surgery, U of T)

Strokes are a source of chronic disability. Effective treatment methods to promote repair of damaged brain tissues remain limited. While transplantation of healthy cells is one potential option to replace damaged tissue following stroke as well as other brain injuries and disease, its effectiveness has not been well demonstrated. Cindi Morshead and her lab have taken a new approach to applying stem cells to repair stroke-damaged tissues. Rather than exogenous stem cell transplantation, they are activating in vivo endogenous repair by stem cells already present in the brain. Administering a drug called Metformin following stroke causes the endogenous stem cells in the brain to proliferate and migrate to the lesion to repair the damage. Morshead and her team found that administering Metformin following a stroke led to reverses in cognitive deficits in mice, potentially offering a new, minimally invasive treatment for stroke and other brain injuries.

### Discovery and Transplantation of *Ex Vivo* Organ Repair Strategies — Shaf Keshavjee (Surgeon-in-Chief, University Health Network)

The scarcity of organs for transplantation is a common issue for transplantations in general, especially lung transplantation. As many as 80 percent of organs are denied at procurement because of poor organ quality and are not suitable for transplant. Dr. Shaf Keshavjee's team has developed and commercialized a novel platform for lung preservation, known as the Ex vivo Lung Perfusion System (EVLP). By perfusing the lung outside the body, nutrients and oxygen can be provided to the lungs and waste products can be removed. This system can preserve lung function for as long as 12 hours before transplantation and has increased the number of successful lung transplantations globally. In addition to preserving organ function, Dr. Keshavjee's team is exploring genetic manipulation of the graft using an adenoviral IL-10 vector to reduce the host's fibrotic response to the graft. This combinatorial therapeutic approach is currently being tested in the clinic, and has the potential to contribute to a new gold standard approach to lung transplantation in the future.

### Trainee Poster Session

More than 50 graduate students and post-doctoral fellows in Medicine by Design-funded laboratories presented research posters. The following trainees received awards:

#### STEMCELL Technologies Awards

- 1st place: Matthew Langley (Peter Zandstra lab) — Boolean network modelling of T cell development predicts heterogeneous single-cell transcriptional trajectories
- 2nd place: Christopher McFaul (Christopher Yip lab) — Light-sheet microscopy of living tissues and organoids
- 3rd place: Yasaman Aghazadeh (Cristina Nostro lab) — Prevascularization of HESC-derived pancreatic progenitors improves graft performance post-transplantation

#### BlueRock Technologies Award

- Nikolaos Mitrousis (Molly Shoichet lab) — Co-transplantation of RPE and photoreceptors rescues vision in a dry AMD mouse model